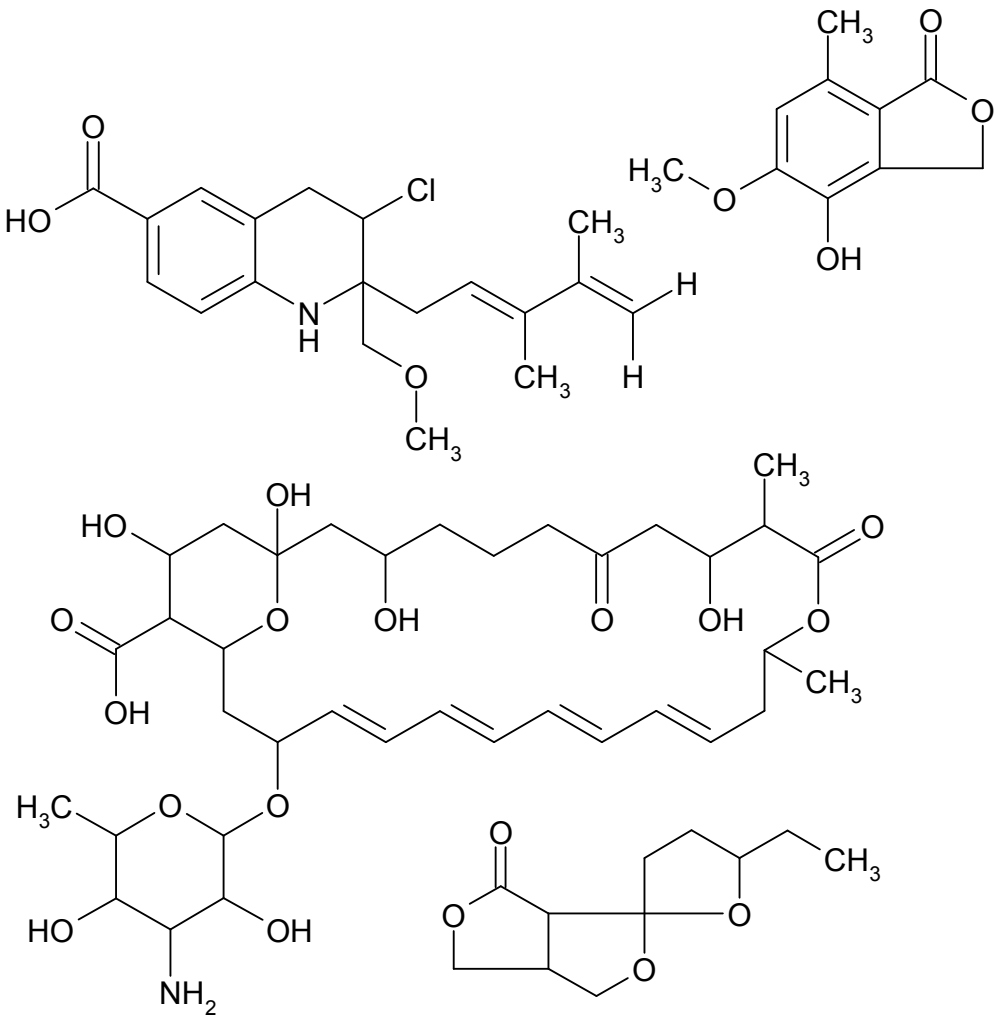
Mahmoud Hussien Ibrahim Al-Refa'i

New and Bioactive Secondary Metabolites from Marine and Terrestrial Bacteria: Ramthacin A, B, C, and Polyene Macrolides from Genetically Modified Bacteria



Dissertation

New and Bioactive Secondary Metabolites from Marine and Terrestrial Bacteria: Ramthacin A, B, C and Polyene Macrolides from Genetically Modified Bacteria

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultäten

der Georg-August-Universität zu Göttingen

vorgelegt von

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aus

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D7

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Korreferent: Prof. Dr. A. Zeeck

Tag der mündlichen Prüfung:

Die vorliegende Arbeit wurde in der Zeit von April 2005 bis Oktober 2008 im Institut für Organische und Biomolekulare Chemie der Georg-August-Universität zu Göttingen unter der Leitung von Herrn Prof. Dr. H. Laatsch angefertigt.

Herrn Prof. Dr. H. Laatsch danke ich für die Möglichkeit zur Durchführung dieser Arbeit sowie die ständige Bereitschaft, auftretende Probleme zu diskutieren.

Für meine Eltern,

meine Frau und meine Kinder

1 Introduction

It is well known that nature has contributed considerable impacts to the drug discovery for human beings in providing remedial treatments since the beginning of human life. The history of drug discovery started from the time man used to take leafs, barks, roots or aerial parts of specific medicinal plants as syrups to treat diseases such as fever, diarrhoea, malaria, vomiting, headache etc. Later, more than 170.000 of natural products have been isolated from plants and other origins, i.e. monoterpenes, sesquiterpenes, tetraterpenes, steroids, alkaloids, flavonoids, coumarins, polyketides, and many others.

During the last decades, natural product chemists turned their interest to search new potential drugs from other sources, such as fungi, algae, cyanobacteria, and bacteria. In the last few years, algae were thought that they were the only organisms using sunlight to grow in the sea, however it was discovered that certain marine bacteria could efficiently exploit solar energy for their reproductive activities as well. Furthermore, in the period of 1882–83 Certes reported firstly deep-sea bacteria.[1] In 1886, another batch sample of bacteria was collected at depths of >1100 m by a passenger ship during a trans-Atlantic cruise.[1]

The medicinal studies of infectious diseases and oncology have gained benefits from numerous drug types derived from natural product sources. Actinomycetes are an example of these natural resources, which has attracted natural product chemists due to their well-developed morphological and cultural characteristics. Previously, they were considered to be an intermediate group between bacteria and fungi, but they were later grouped as Gram-positive bacteria with branched filaments. Due to the ability to produce bioactive secondary metabolites and enzymes, actinomycetes from different marine and terrestrial environments have gained interest in the study of their biodiversity. Actinomycetes are significant antibiotic producers contributing three quarters of all known microbial products. Streptomycetes are a sub-class of actinomycetes, which have contributed over 80% of the total antibiotic products isolated in addition to other classes of biologically active secondary metabolites. They are aerobic bacteria and are found mainly in soil samples and compost. They form aerial mycelia and give off an "earthy" odour.

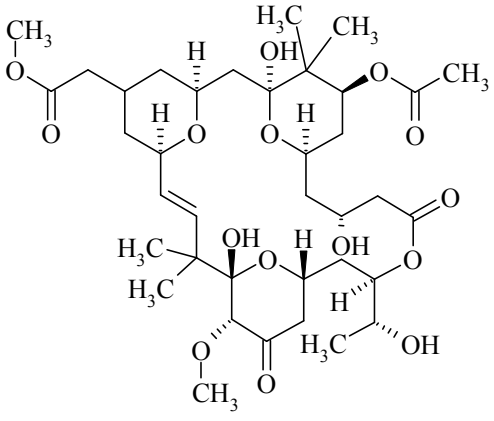
Many classes of specific bioactivities have been found in marine natural products. These include anticancer, antibiotics, antifungal, antiviral, antiplasmodial, antiparasitic, tumour-promoting, immune suppression, antileukaemic, anti-inflammatory, antitubercular, and antitumor activities. Furthermore, a high structural variety has been

reported from marine organisms, such as bioactive peptides, heterocyclic alkaloids, sulphur-containing compounds, polyethers, macrolides, sesquiterpenoids and diterpenoids, etc.

1.1 Anticancer agents

The contribution of natural products to pharmaceutical industries has been reviewed recently.[2] Many important compounds with high bioactivities have been introduced especially as cancer chemotherapeutic. Currently, research in the area of anticancer drugs is focusing on interesting mechanisms of action and corresponding signal transduction and kinases.[3] New classes of antitumor agents have established new opportunities in cancer treatment. Among these, bryostatin 1 (**1**), which was isolated from a marine animal (but is originally synthesized by endosymbiotic bacteria), has exhibited fascinating results on protein kinase C (PKC), and consequently, affects signal transduction pathways within cells.[4,5] Fumagillin (**3**) analogues, which have been illustrated as a new generation of anticancer drugs showed interesting antitumor activity. These compounds did not affect cancer cells but they affected the tumor neovascularisation.[5] More recently, cyclostreptin (FR182877, **2**) isolated from *Streptomyces* sp. was found to be a very good inhibitor of the growth of human cancer cells.[2]

Another series of active compounds with novel structures are epothilone B (**4**), eleutherobin (**5**) and discodermolide (**6**), which are now under clinical trials as anticancer agents. These compounds act with the same basic mechanism as taxol (**7**), an amazing anti-cancer drug, currently used against ovarian and breast cancer.[6]



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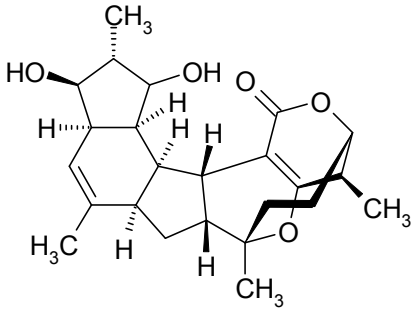

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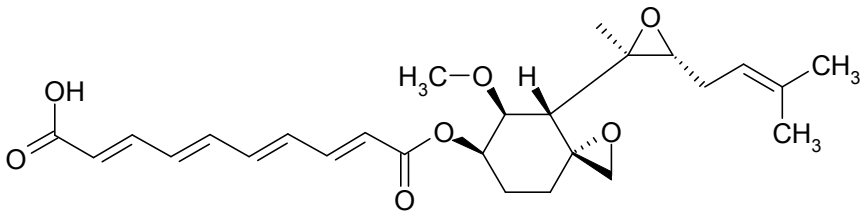

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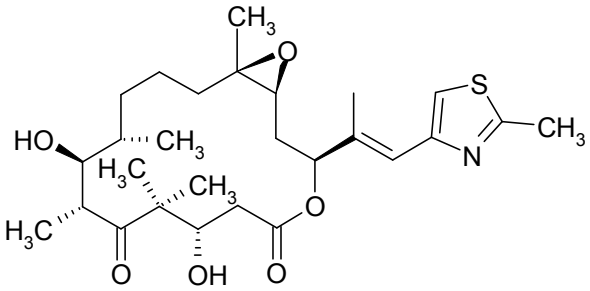

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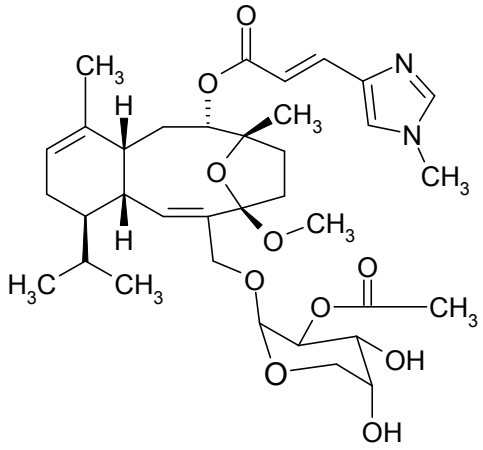

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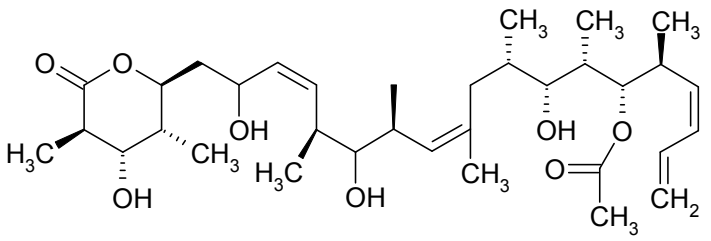

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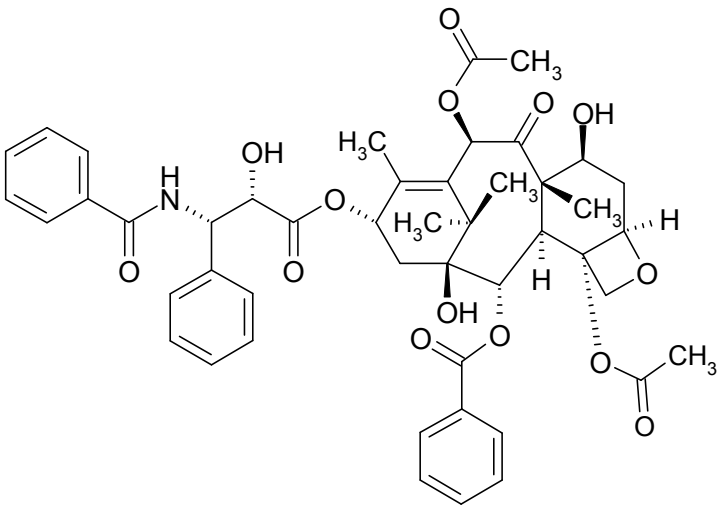

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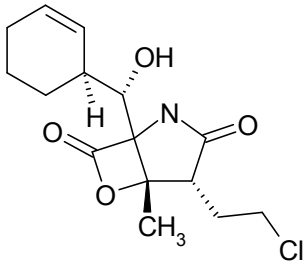
The anti-cancer salinosporamide A (NPI-0052) (**8**) from marine bacteria has bio-activity as potent killer of drug-resistant multiple myeloma cells. This type of cancer has been reported as hopeless disease attacking the bone marrow, in which both red

and white blood cells are produced. Multiple myeloma is considered to be the second most common type of blood cancer.[7] Compound **8** exhibited less toxicity than other chemotherapeutic drugs.



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Three novel aminofuran antibiotics, proximicin A (**9**), B (**10**), and C (**11**) with anticancer activity, were recently isolated from a marine *Verrucosispora* strain MG-37. The cytostatic effect of these three compounds was tested in different tumor cell lines and showed significant growth inhibitory activities towards gastric adenocarcinoma (AGS) and hepatocellular carcinoma (Hep G2). Unfortunately breast carcinoma cells (MCF 7) were less sensitive.[8]

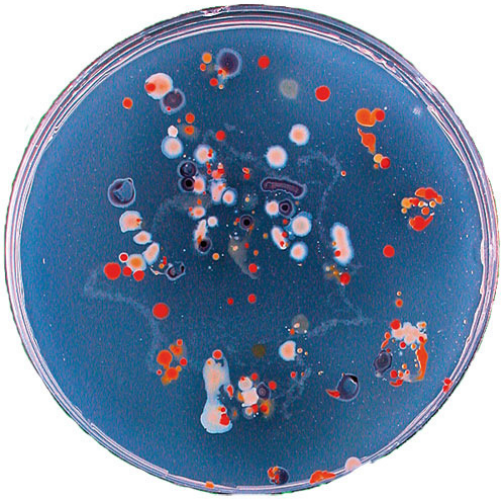
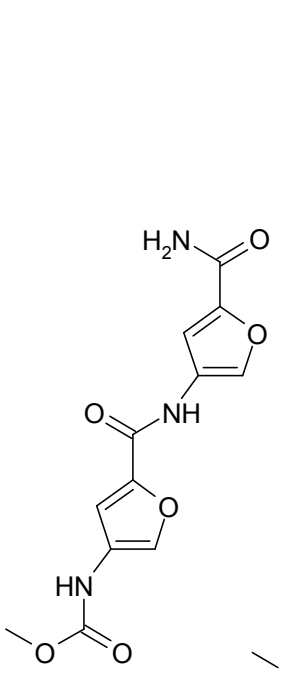


Figure 1. A diversity of marine bacteria is a supply of novel structures.[9]



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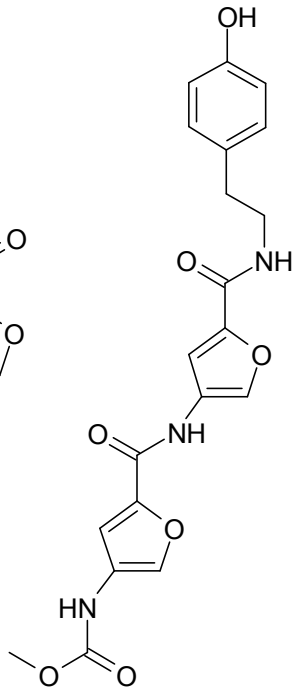

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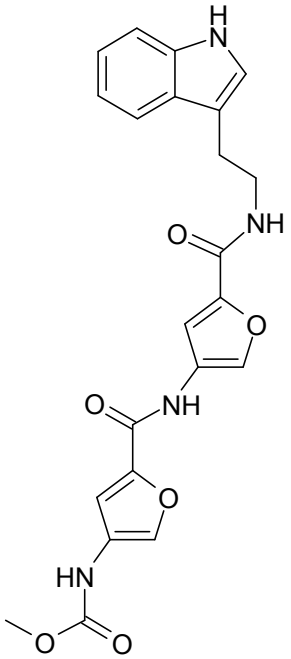

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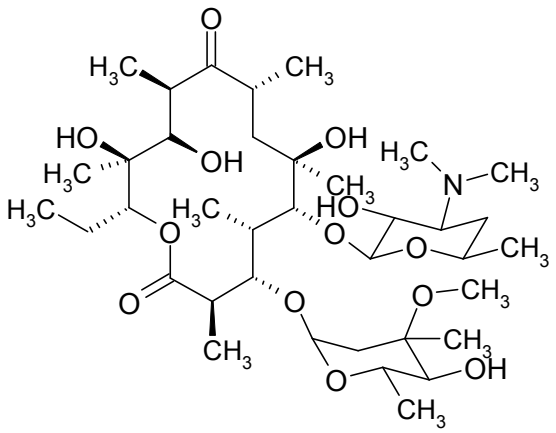
1.2 Antibiotics

Erythromycin (**11**) as a typical example of macrolide antibiotics has been utilized since the 1950s in treatment of infections with Gram-positive bacteria, however it showed relatively poor clinical potency against *Mycobacterium tuberculosis*. A series of 3, 6, 9, 11, or 12-substituted analogues of erythromycin were tested *in vitro* and *in vivo* against *M. tuberculosis*. The results indicated that only the 11,12-carbazate derivatives (cyclic 3-amino-oxazolidine-2-one derivatives) of 3-keto-6-*O*-methylerythromycin A (**13**)[10] showed activity against *M. tuberculosis in vivo*.[11]



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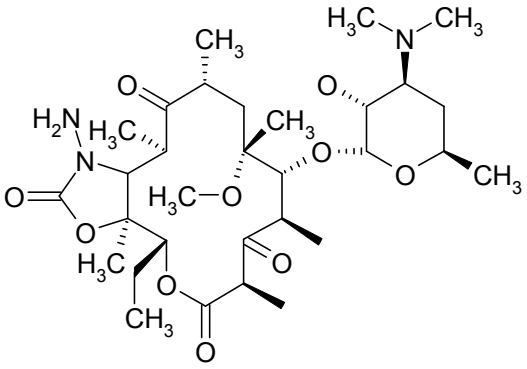

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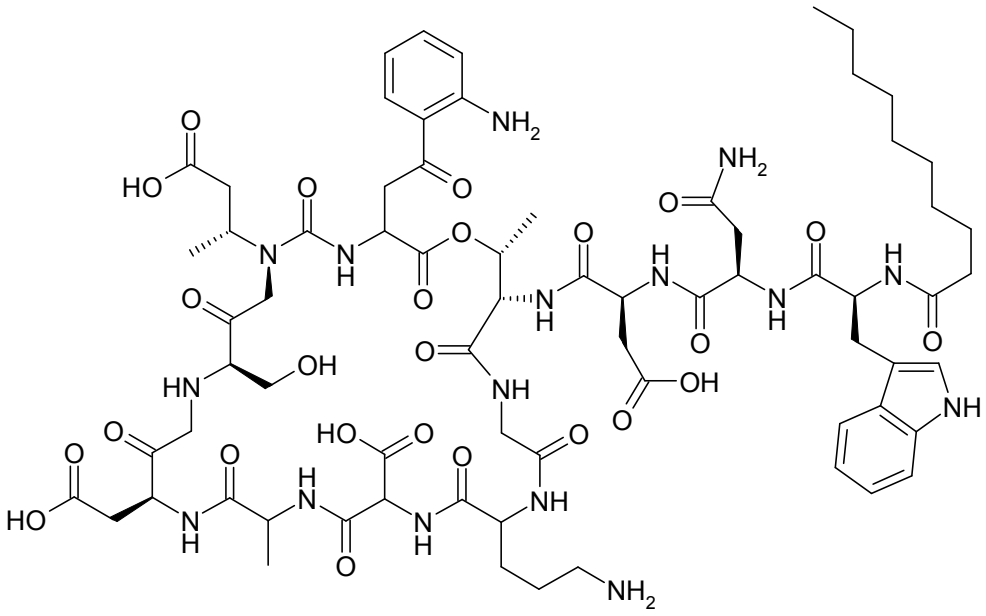
Although the antibacterial spectrum of macrolide antibiotics against Gram-positive bacteria is narrower than that of penicillins, they contribute a second antibacterial alternative for penicillin intolerant patients. The ketolides, another class of macrolide antibiotics, are used to treat respiratory tract infections due to macrolide-resistant bacteria.

Recently, the study of biosynthetic gene clusters of natural bioactive compounds from old sources such as *Streptomycetes* as well as new sources such as cyanobacteria, resulted in the discovery of numerous new potential antibiotics classes with novel mechanisms of action. Daptomycin (cubicin, **14**) has been introduced for human use to treat bacterial infections. The biosynthetic gene clusters for daptomycin and related acyldepsipeptidolactones have been sequenced, and combinatorial biosynthesis has been performed to provide a potential pathway to the second-generation daptomycins.



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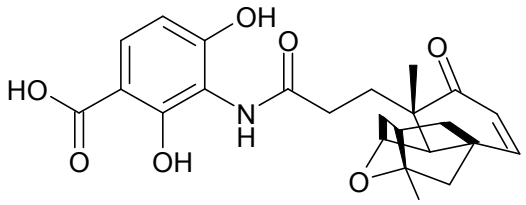
Platensimycin (**15**), which has been isolated independently in our group,[12] has demonstrated strong broad-spectrum antibacterial activity by inhibiting cellular lipid biosynthesis. Similarly, cerulenin and thiolactomycin have been also reported to inhibit condensing enzymes in the fatty acid biosynthesis. Platensimycin showed specific and selective activities against the Fab/F enzyme in the fatty acid biosynthesis pathway. Due to its unique mode of action, platensimycin displayed no cross-resistance to methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and other tested antibiotic-resistant strains.[13]



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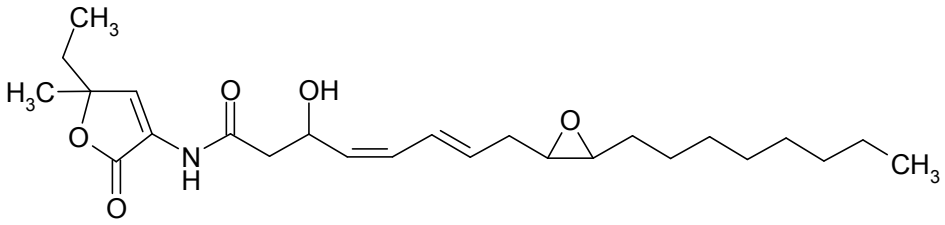
Korormicin (**16**), which was isolated from the marine bacteria, *Pseudoalteromonas* sp. F-420 has been reviewed as novel antibiotic. The compound showed specific inhibitory activity against marine Gram-negative bacteria, but interestingly, was inactive against terrestrial microorganisms.[14]



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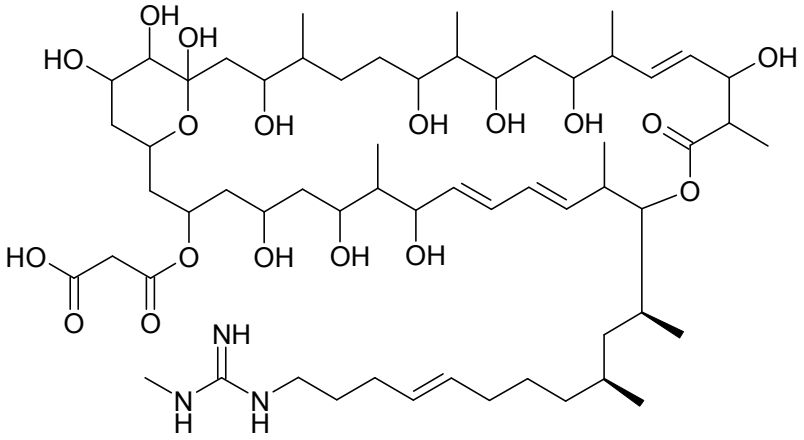
Niphimycin (**17**) is a polyol macrolide antibiotic, which was first isolated from a strain of S*treptomyces hygroscopicus*. This compound showed broad antimicrobial activities against Gram-positive bacteria as well as yeast and fungi, while amphotericin B, which is a polyene macrolide having many hydroxy groups showed activity against fungi.[15]



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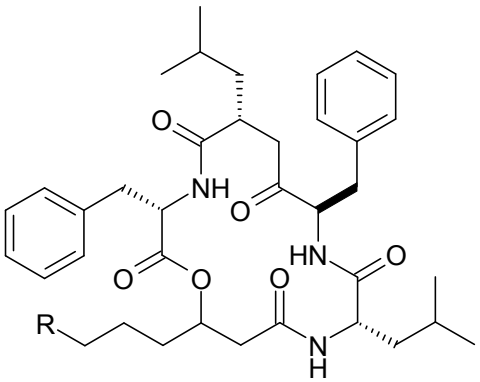
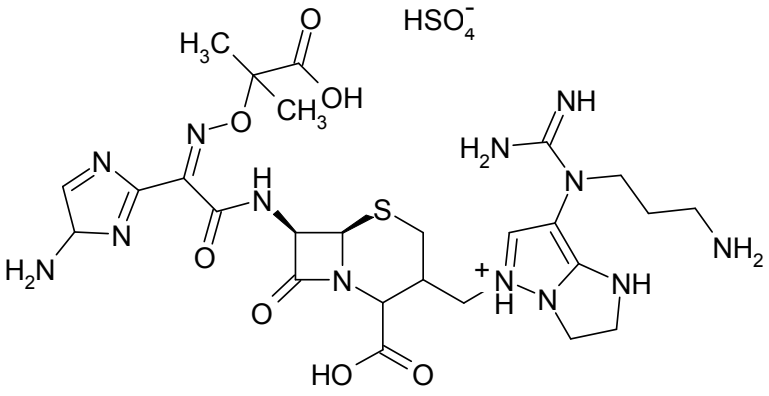
Unnarmicin C (**18**) and A (**19**), two depsipeptide antibiotics, were isolated from the marine bacterium, *Photobacterium* sp. strain MBIC06485. They exhibited selective inhibition against two strains of *Pseudovibrio* sp.[16] Also reported was a novel dihydroimidazopyrazolium cephalosporin FR295389 (**20**), which has proven to be effective against IMP-type metallo-*β*-lactamase (MBL) producing *Pseudomonas aeruginosa* even though previously reported cephalosporins have been shown to be ineffective towards MBL-producing strains.[17]



18 R = Et **19** R = H



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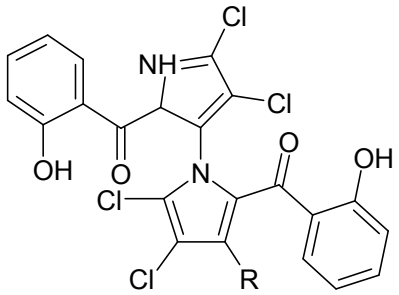
Other examples are the uncommon bispyrrole antibiotics, which were isolated recently from a deep sea marine *Streptomyces* strain. Marinopyrrole A (**21**) and B (**22**) exhibited interesting antimicrobial activity against drug-resistant bacteria, especially methicillin-resistant *Staphylococcus aureus*, and both compounds showed minimum inhibitory concentrations (MIC_{90}) of less than 2 μM.[18]



21 R = H, 22 R = Br

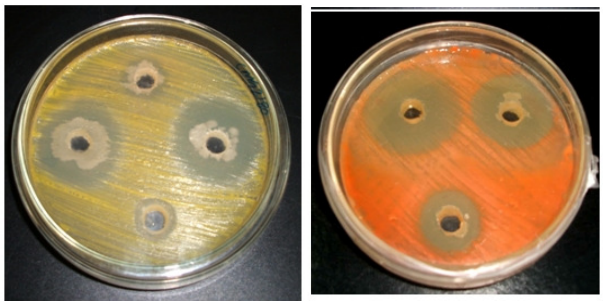
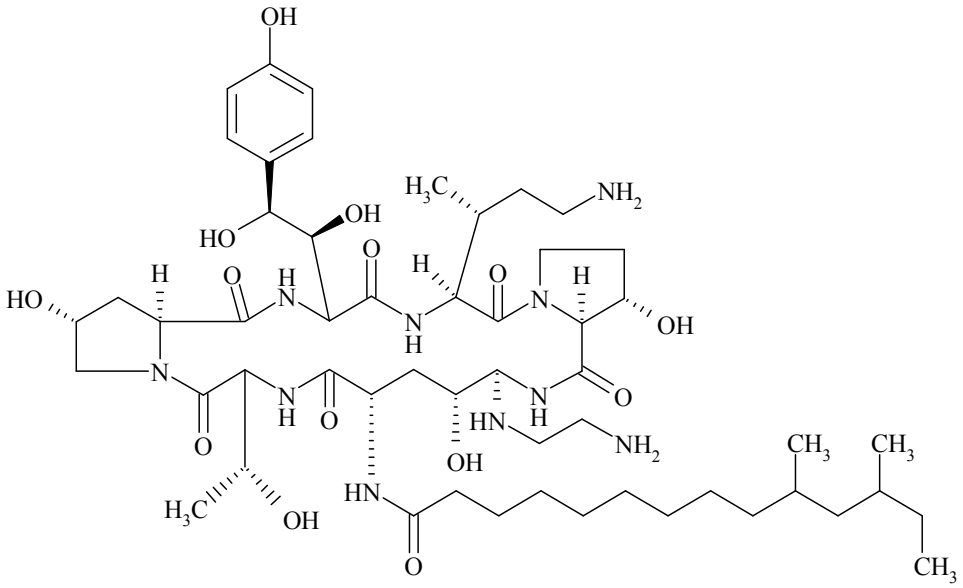


Figure 2. Bioactivity of natural products isolated from marine bacteria[19]

1.3 Antifungal agents

A continuous search for new anti-fungal agents from different microorganisms has been an ongoing process in order to find specific drugs to treat widespread infections. Nature offers a virtually unlimited source of unique molecules, which can act as a reservoir for potential drugs. Currently, caspofungin, (**23**)[20] has been reported as the most common clinical anti-fungal agent. It was derived from pneumocandin, a metabolite produced by *Glarea lozoyensis.*



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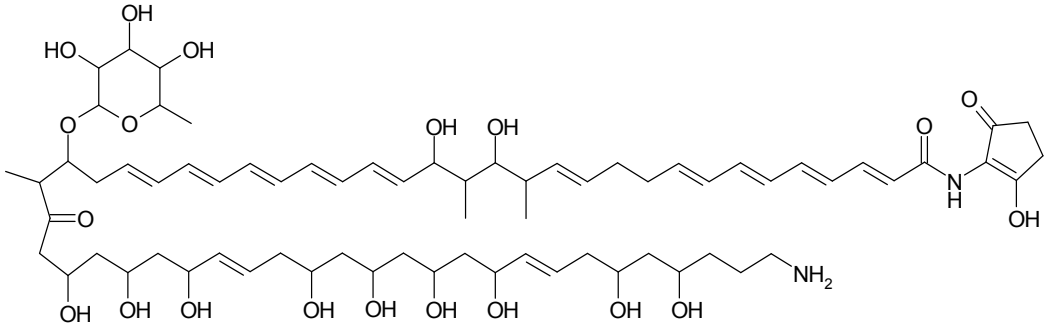
By using a genomic approach, the discovery of the anti-fungal natural product ECO-02301 (**24**), was successfully carried out. Moreover, analysis of the genome of *Streptomyces aizunensis* NRRL B-11277 indicated the potential to produce a molecule with a novel and highly predictable structure. ECO-02301 (**24**) showed potent anti-fungal activity against a variety of human-pathogenic fungi.[21]



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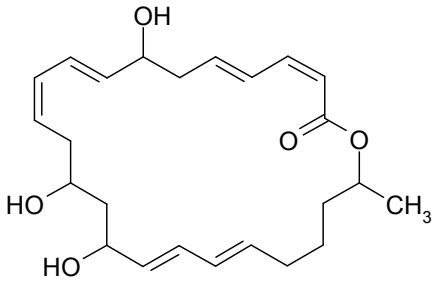
1.4 Antiviral agents

Currently, HIV infections are treated by synthetic compounds, which are available on the markets. In recent years natural products have been utilized for AIDS therapy, and betulinic acid derivatives are excellent examples. These compounds have been assessed as first-class HIV inhibitor and are recently in phase II clinical trial.

Asteropine, which was isolated from a marine sponge, is known as antibacterial agent. However, asteropine analogs have demonstrated also anti-viral activity.[22] Another example is macrolactin A (**25**), which was isolated from a deep-sea marine bacterium. These types of compounds showed significant inhibition of mammalian *Her-*

pes simplex viruses (types I and 11) with IC_{50} values of 5.0 and 8.3 μg/mL, respectively, and protected T-lymphoblast cells against human HIV viral replication.[23]



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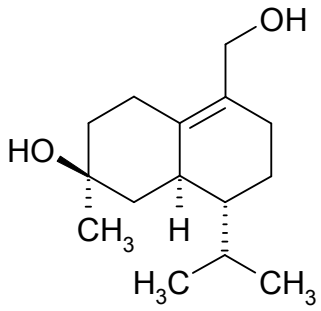
Additionally a cadinane sesquiterpene, 4β,14-dihydroxy-6α,7*βH*-1(10)-cadinene (**26**), was isolated from the cultures of the basidiomycete, *Tyromyces chioneus*, which also showed significant anti-HIV-I activity.[24]



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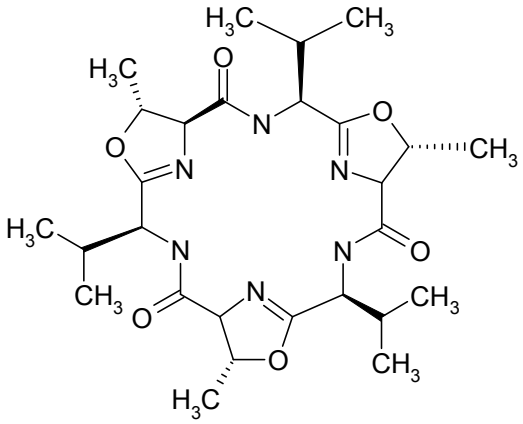
1.5 Cyanobacteria as a source of natural products

At least 990 novel secondary metabolites have been isolated from blue green algae. The ability to provide cytotoxic nonribosomally derived peptides and depsipeptides has been investigated from terrestrial cyanobacteria. At least 16 distinct modular NRPS/PKS clusters of the cyanobacterium *Nostoc punctiforme* have been reported based on genome analysis. Blue-green algae have been reviewed as new source of unique and bioactive peptides and polyketides. Furthermore, both molecular genetic studies and biosynthetic experiments with labelled precursors, have increased the possibility to culture several species of blue green algae, especially of the genus *Lyngbya*. Westiellamide (**27**) and scytophycin C (**28**) were obtained from a terrestrial cyanobacterium, while cryptophycin-24 (**29**) was produced by a terrestrial *Nostoc* sp. Interestingly, the *Nostoc* sp. 53789, which was isolated as a symbiont of a lichen, produced cryptophycins. Nodularin (**30**) was another novel structure isolated from *Microcystis aeruginosa* and *Planktothrix agardhii*.[25]



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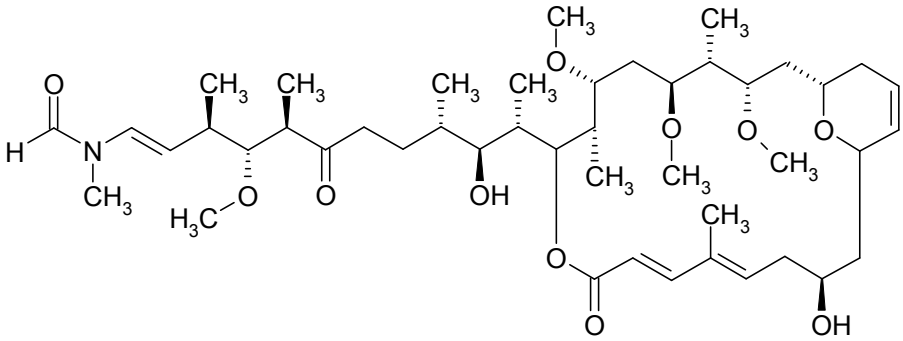

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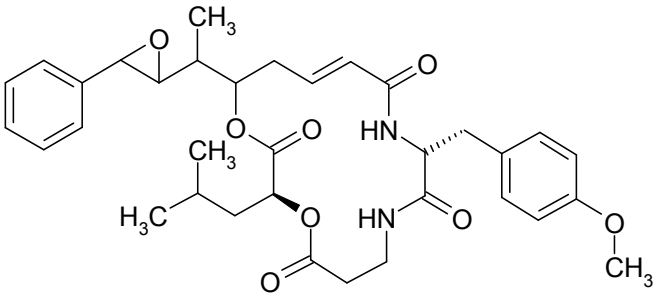

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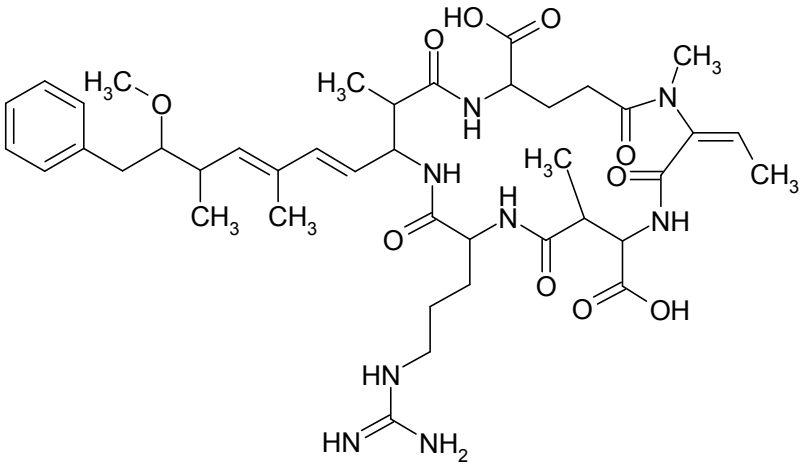

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2 Scope of the present study

Cyanobacterial toxins represent an increasing risk for the health of wild and domestic animals, but also for humans. About 1000 natural products have been isolated so far from these organisms, many of them with structures, which do not resemble natural products from other sources. Compared with the estimated number of these blue-green algae still to be explored, this seems still to be little. Further investigation of cyanobacteria and their toxins is of high interest from a medical and ecological point of view, especially in developing countries like Jordan. However, there are many chemical questions and problems of basic research still to be answered. There is much speculation about whether the cyanobacteria itself or the associated microorganisms are responsible for this high diversity of metabolites, and therefore the chemical diversity is certainly higher than presently known.

A theme of this thesis was therefore the investigation of cyanobacterial algal blooms from Jordan and – if possible – comparison with metabolite patterns from axenic cultures. This task was planned together with local co-operation partners in Jordan who were responsible for harvesting and culturing the cyanobacteria. Industrial partners would perform the important taxonomic determinations.

As algal blooms usually occur only in short periods of the year and are not predictable, and as the mass-cultivation of cyanobacteria is a slow and difficult process, streptomycetes should be investigated in parallel, to make best use of the time. Although streptomycetes have been intensively investigated, they continue to be a prolific source of novel and biologically active metabolites. It was therefore predictable that the cultivation of terrestrial or marine strains would easily deliver new compounds of interest and thus would reduce the risk of handling the cyanobacteria.

To achieve this purpose, chemical (TLC/ HPLC-MS) and biological screening for the desired strains had to be done in a sequence as outlined in the following steps:

To investigate the chemical constituents of endemic Jordanian cyanobacterial flora, with respect to medical and ecological implications, a broad biological and chemical screening of Jordanian strains needs to be performed. In a second step, selected strains will be upscaled to gain sufficient cell material for chemical investigation.

The microbial extracts obtained from the fermentation broth or algal blooms will be subjected to different chromatographic methods (i.e. HPLC, Sephadex, silica gel column chromatography, PTLC, etc.) to attain pure metabolites. The isolated compo-

nents will be measured spectroscopically and known compounds identified by searches in databases (AntiBase, Dictionary of Natural Products, and Chemical Abstracts) for dereplication. The final structure elucidation of new compounds should be done by MS and 2D NMR measurements (^{1}H,^{1}H COSY, HSQC and HMBC).

Finally, the isolated new and pure metabolites should be tested for their biological activites through different bioassays (i.e. antimicrobial test and brine shrimp assay, and receptor tests by cooperation partners).

3 Cyanobacteria

In a German/Jordanian cooperative research project funded by the Deutsche Forschung Gemeinschaft (DFG), four Jordanian cyanobacteria strains were considered for further chemical and biological investigations. For chemical and biological screening purposes, these strains were cultured in small scale at the Al-al-Bayt University, Jordan. The four strains were *Nostoc linkia*, *Nostoc commune*, *Anabaena* pcc7120 and a *Scytonema* strain. The extracts from *Nostoc commune* and *Anabaena* pcc7120 were active against *Staphylococcus aureus*, *Bacillus subtilis* and *Streptomyces viridochromogenes* (Tü 57). Toxicity tests were performed using the brine shrimp assay, but none of the extracts showed positive results.

The strains *Nostoc linkia*, *Nostoc commune*, *Anabaena* pcc7120 and a *Scytonema* strain were obtained from Prof. Alani, isolated by Prof. Al-Mahasnih from Al al-Bayt University, and identified by AnagnosTec GMbH, Luckenwalde, Germany. A *Microcystis aeruginosa* strain was isolated and identified by Prof. S. Al-Jasabi and Prof. A. Khalil from Yarmouk university, the strain was collected from king Talal dam by Dr. M. Halawa Pheladelphia university, Jordan.

3.1 Scytonema sp.

MALDI-TOF MS measurements were performed to obtain a fingerprint of all the masses (in the range of 500 to 20,000 Dalton) present in the crude extracts (Figure 3). The extract of the *Scytonema* strain comprised of seven major compounds with masses ranging from *m/z* 535 to 1045 of which a component at *m/z* 995 was most interesting. Based on a search in the SARAMIS-Database using the spectroscopic data, no hits were obtained for this component, which suggested that this was a new microcystin. Additionally two other peaks were observed with molecular weights *m/z* 593 and 871, which were identified as pheophytin a (**31**) and pheophobide a (**32**), respectively.

Since peptides of cyanobacteria were the main focus of the research project and microcystins are of special interest due to their toxicity, this strain was considered for further research.

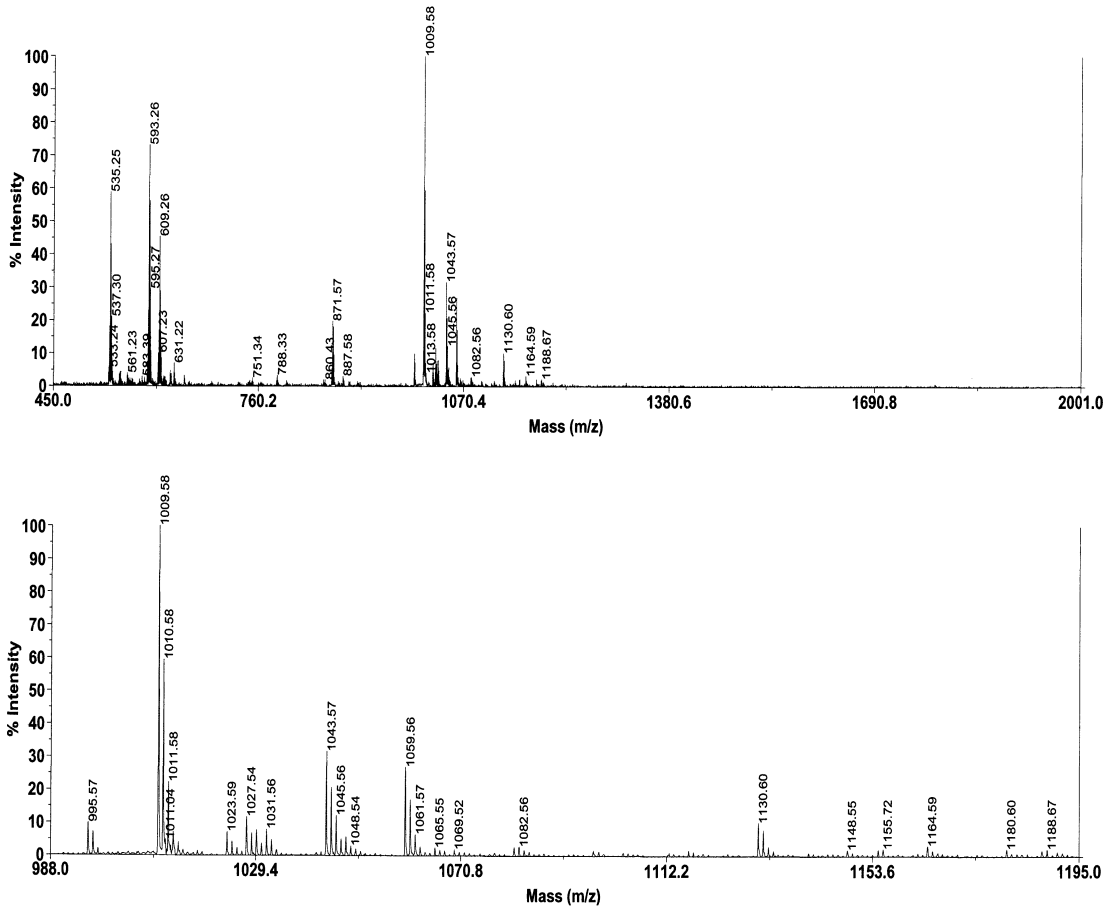
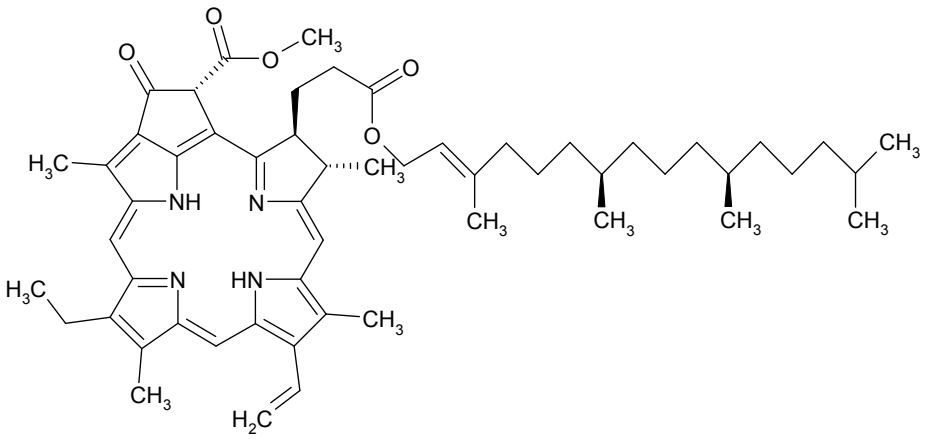


Figure 3. MALDI-TOF mass spectrum of the *Scytonema* sample.



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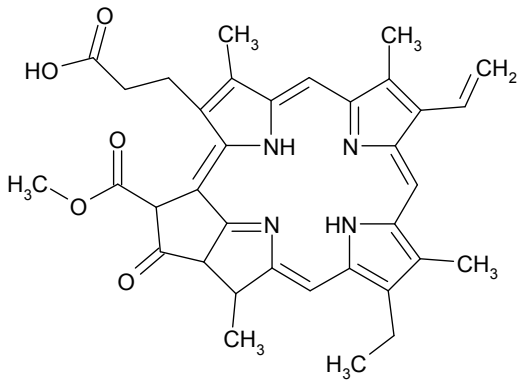

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The *Scytonema* strain was cultivated again using the Chu's medium 10. An amount of 7.50 g (dry weight) of cell material was obtained, which was extracted with dichloromethane/methanol (1:1) followed by methanol to afford approximately 0.40 g of crude extract. This material was subjected to the usual separation procedures like chromatography on silica gel, Sephadex LH-20 and by HPLC-ESI-MS/MS, NMR spectroscopy and other analytical methods to elucidate the structures.

The crude extract consisted mostly of fatty acids and glycolipids. The concentration of the new microcystin (*m/z* 995) was very low and as a result, attempts to isolate and characterise this compound using HPLC-ESI-MS/MS was not successful. Only pheophorbide A and pheophytin A along with oleic acid were characterised from this strain. There were also carotenoids present in this strain, but their stability and amounts were insufficient for characterization.

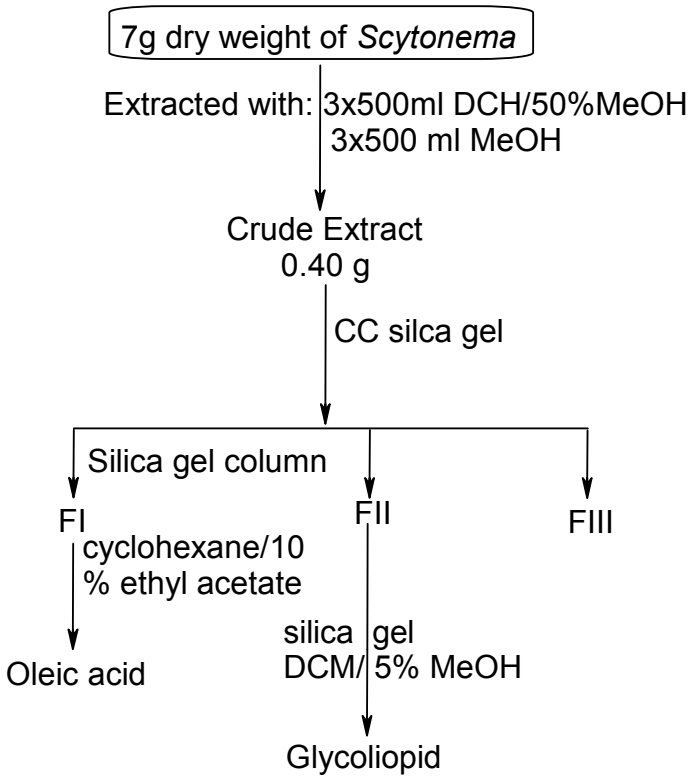


Figure 4. Work up scheme of *Scytonema*.

3.1.1 3-O-β-D-Galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)glycerol and Diglycolipid 16

A mixture of **33** and **35** and was isolated as colourless oil from fraction II (Figure 4) by silica gel column chromatography. It was UV absorbing at 254 nm and turned black with anisaldehyde spray reagent. The ^{1}H NMR spectrum indicated the presence of two olefinic proton signals at δ 5.35 (t, H-9', 10'), one oxy-bearing methine at 5.30 (H-2) and two ABX methylene groups CH_2-1 (dd, δ 4.47, 4.21) and CH_2-3 (dd, δ 3.98, 3.73). In the ^{1}H NMR spectrum, sugar signals were visible at δ 4.22-3.75. In addition, four methylenes connected to sp^2 carbon atoms at 2.33 (td, 4H, H-2', 2") and 2.00 (m, 4H, H-8", 10"), further methylene signals between 1.40-1.22 as well as to two methyl groups at 0.88 (t, 6H, H-15', H-15") were observed.

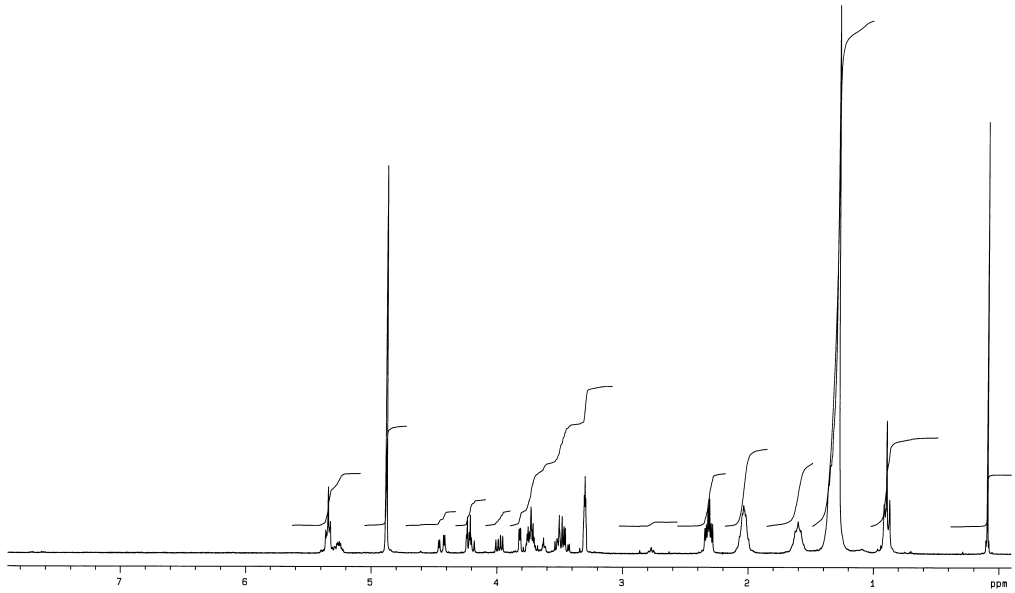
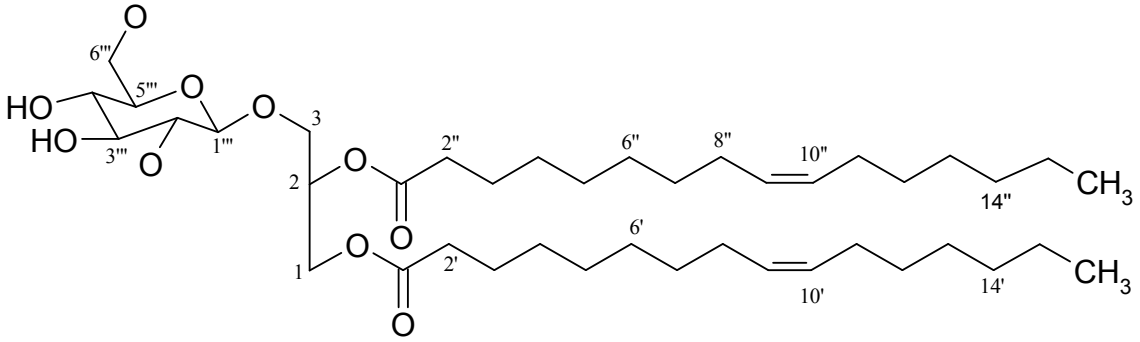


Figure 5. ^{1}H NMR spectrum (300 MHz, CD_3OD) of 3-O-β-D-galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)glycerol (**33**) and diglycolipid 16 (**35**).

ESIMS analysis in the positive mode gave two *pseudo*molecular ion peaks at *m*/*z* 751 ($[M_1 + Na]^+$) and 749 ($[M_2 + Na]^+$), which indicating a mixture of two compounds with 2 amu difference pointing to one double bond more. (+)-HRESIMS for M_2 at *m*/*z* 744.56215 ($[M_2 + NH_4]^+$) suggested a molecular formula of $C_{41}H_{74}O_{10}$. Searching in AntiBase[26] using the above spectroscopic data resulted in diglycolipid 17 (**34**) and 3-O-β-D-galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)-glycerol (**33**) as two possible structures for the mass M_2 (726 Dalton), and diglycolipid 16 (**35**) for the mass 728. The structure of **33** and **35** were confirmed by the ESI-MS/MS measurements (Figure 7, Figure 8).

ESI-MS^2 of the molecular ion *m*/*z* 751 ($[M_1 + Na]^+$) delivered one major fragment at *m*/*z* 495 due to the loss of 9-*cis*-hexadecenoic acid (*m*/*z* 254). Structure **34** was eliminated due to the absence of fragments at *m*/*z* 493 and *m*/*z* 497, which would be expected from the loss of hexadecenoic acid (*m*/*z* 256) and (9Z,12E)-9,12-hexadecadienoic acid (*m*/*z* 252), respectively. However, $ESIMS^2$ of the molecular ion *m*/*z* 749 ($[M_1 + Na]^+$) showed two fragments at *m*/*z* 495 and 497 due to the alternate loss of two fatty acid residues hexadecenoic acid (*m*/*z* 256) and 9-*cis*-hexadecenoic acid (*m*/*z* 254).



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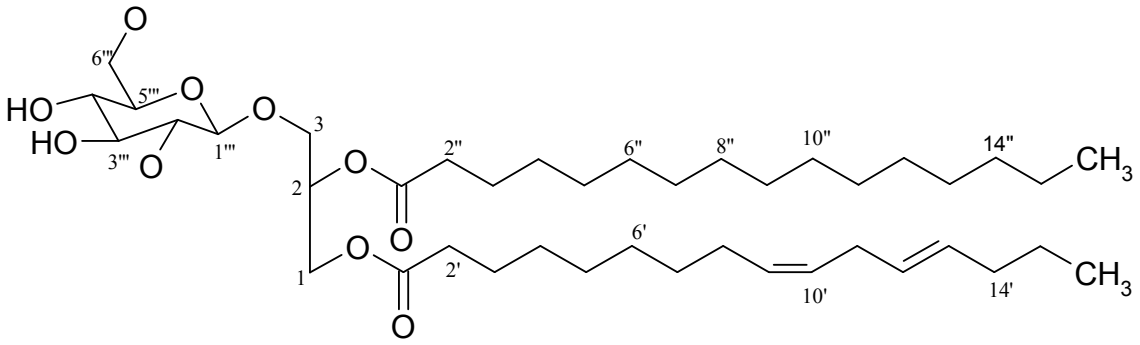

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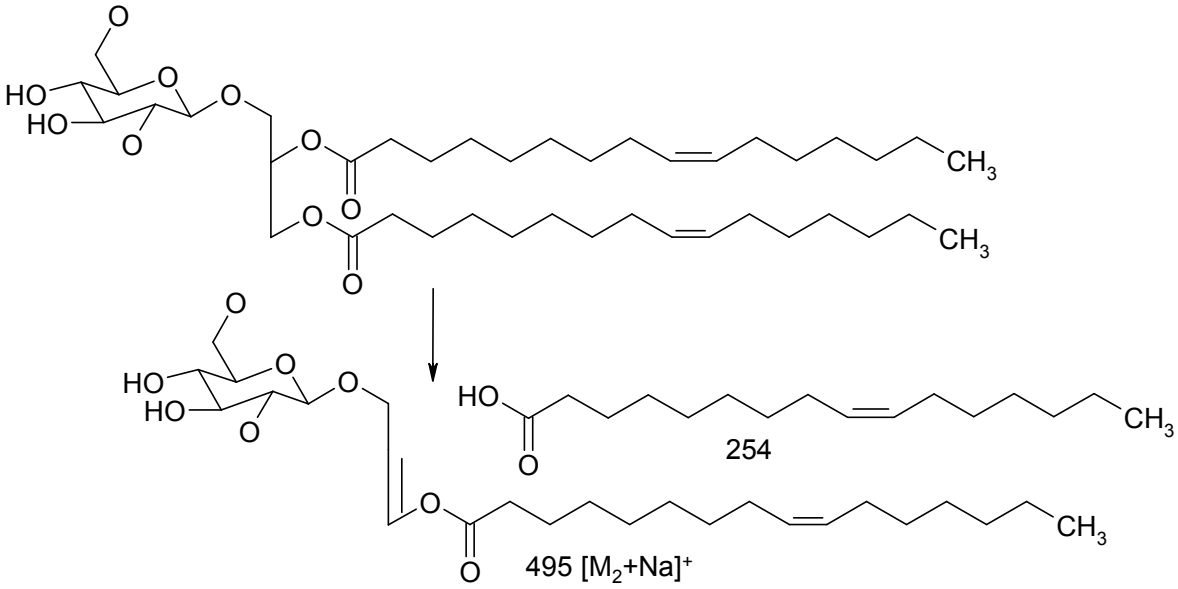


Figure 6. Fragmentation pattern observed in the ESI/MS/MS spectrum of 3-O-β-D-Galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)glycerol (**33**).

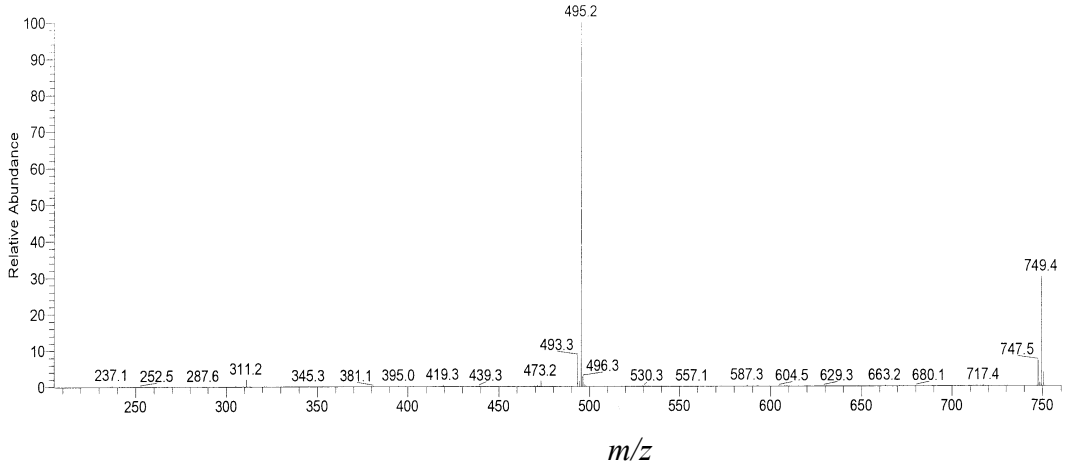
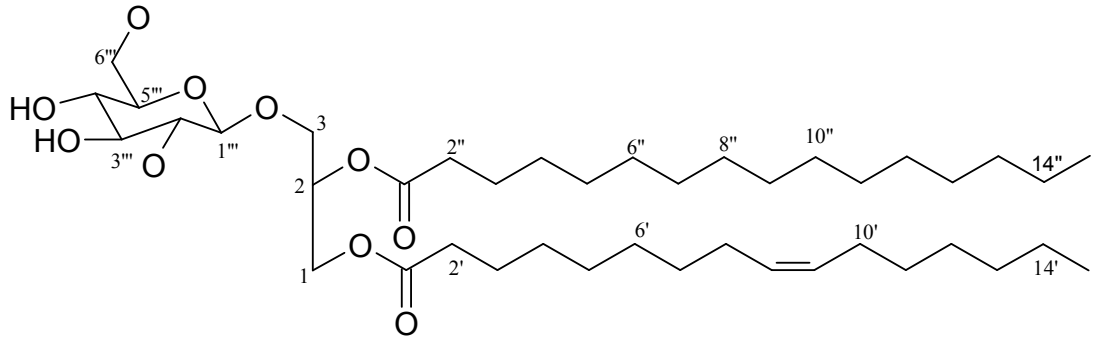


Figure 7. The MS^2 of *m/z* 751 $[M_1+ Na]^+$ of 3-O-β-D-galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)glycerol (**33**).



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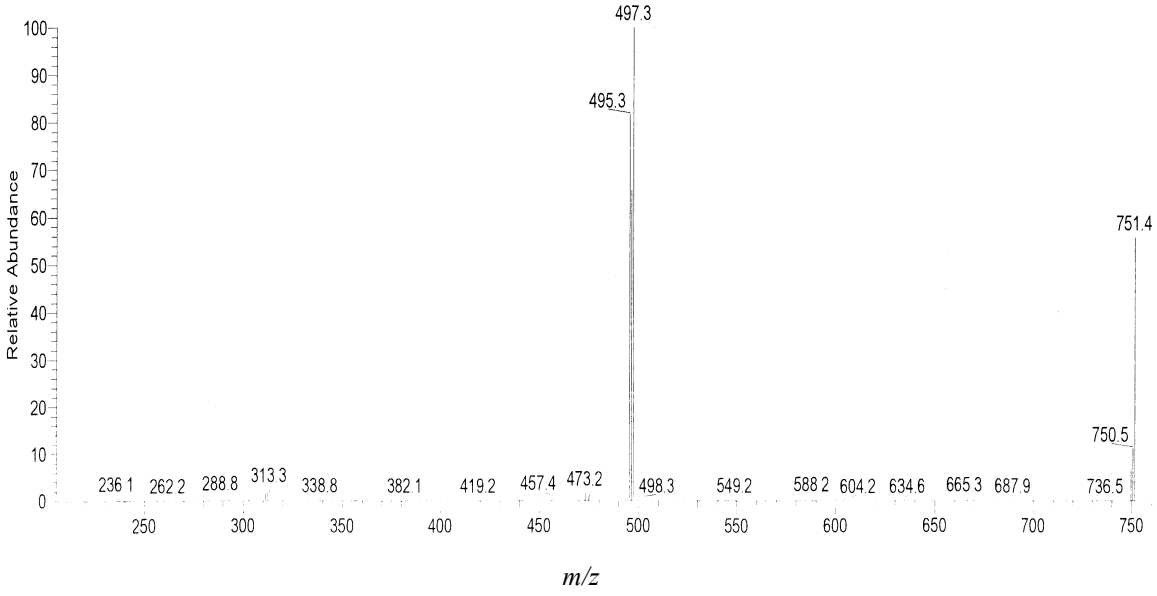


Figure 8. The MS^2 of *m/z* 749 $[M_2 + Na]^+$ of diglycolipid 16 (**35**).

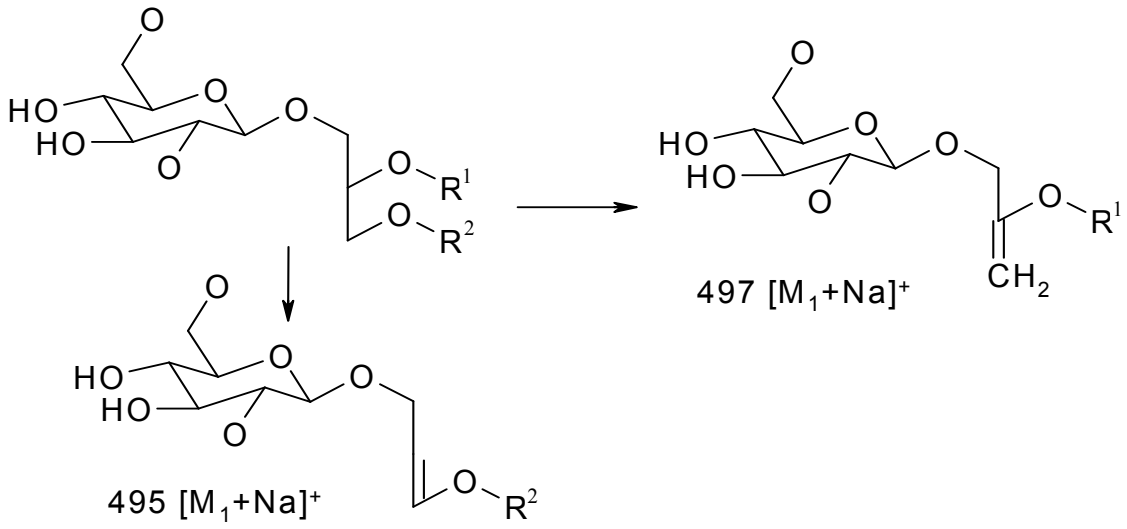


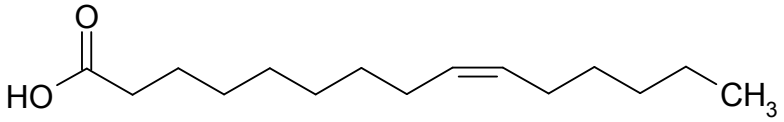
Figure 9. Fragmentation pattern observed from the ESI/MS/MS spectrum of diglycolipid 16 (**35**).

Glycolipids are well-known in photosynthetic eucaryotic[27] and prokaryotic[28] organisms, where they are linked to the thylakoid membranes. In cyanobacteria glycolipids are also associated with the heterocystous cell walls.[29,30] Glycolipids are reported as having specific biological activities e.g., antitumor-promoting,[31] anti-inflammatory,[32] antialgal[33] and antiviral.[34,35] Compound **33** was isolated from the marine bacillariophycean microalga *Nitzschia* sp. in 2001 by Son *et al*.[36] Diglycolipid 16 (**35**) was isolated from the cyanobacterium *Scytonema* sp. (TAU stain SL-30-1-4) and found to inhibit HIV-1-RT enzymatic activity.[37]

3.1.2 Oleic acid

Compound **36** was isolated from fraction I as colourless, non-UV absorbing oil, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum exhibited an exchangeable proton signal at δ 11.50, a multiplet signal at δ 5.38 of two olefinic protons (H-9, 10), three methylene signals connected to sp^2 carbons at δ 2.36 (t, CH_2-2), 2.00 (m, CH_2-9, 10), as well as seven methylenes at δ 1.30. Additionally, one methyl triplet was observed at δ 0.87. With these data and by comparison with reference spectra, this compound was identified as oleic acid (**36**).

In addition to oleic acid, two saturated fatty acids, pentadecanoic acid and octadecanoic acid were characterized as methyl esters by GC-MS as well as by gas chromatographic comparison with authentic standards.



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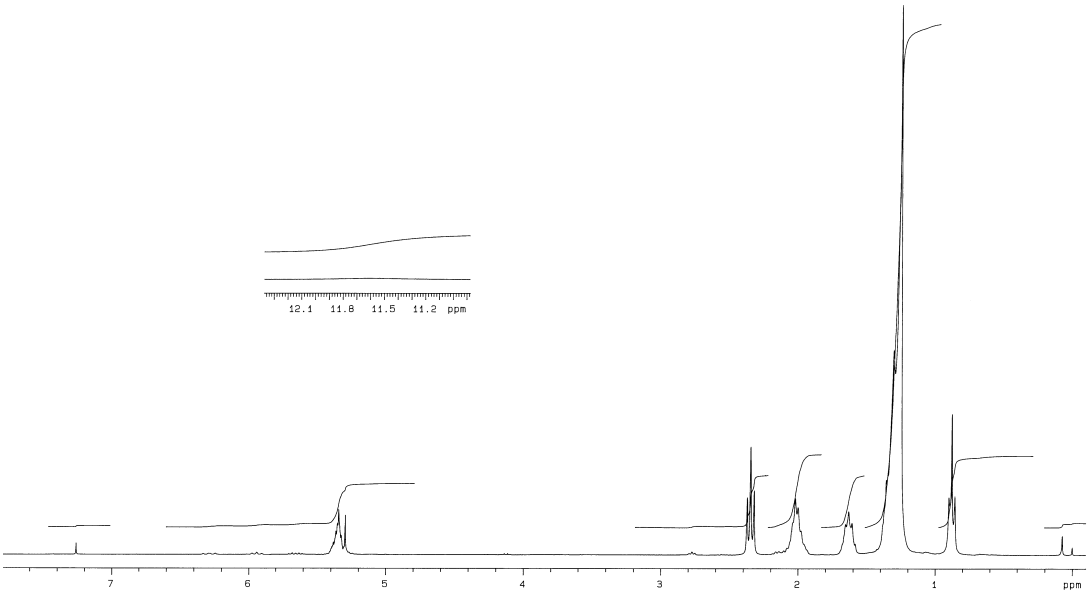


Figure 10. ^{1}H NMR spectrum (300 MHz, CH_2Cl_2) of oleic acid (**36**).

Oleic acid is the main monounsaturated fatty acid of olive oil: It suppresses Her-2/neu over-expression, which synergistically interacts with anti-Her.2/neu immunotherapy by promoting apoptotic cell death of breast cancer cells with Her-2/neu oncogene amplification.[38] In addition, it was reported that oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells.[39]

3.2 Microcystis aeruginosa

Cyanobacteria produce two main groups of neurotoxins and peptide hepatotoxins, which cause poisoning of wild and domestic animals and are a risk for the human health in many parts of the world. *Microcystis aeruginosa* is the most common toxic cyanobacterium in the eutrophic freshwater; its cells can join together in groups as colonies which help this species to float near the water surface.[40] The species investigated here was collected form king Talal damp in Jordan by Prof. M. Halawa. The sample was subjected to MALDI-TOF MS experiments (Figure 11), which revealed three major compounds with masses *m/z* 973, 995 and 1045 respectively. These were characterised as microcystins based on a search in the SARAMIS-Databank using the spectroscopic data pointing to the compound at *m/z* 973 as possibly new derivative.

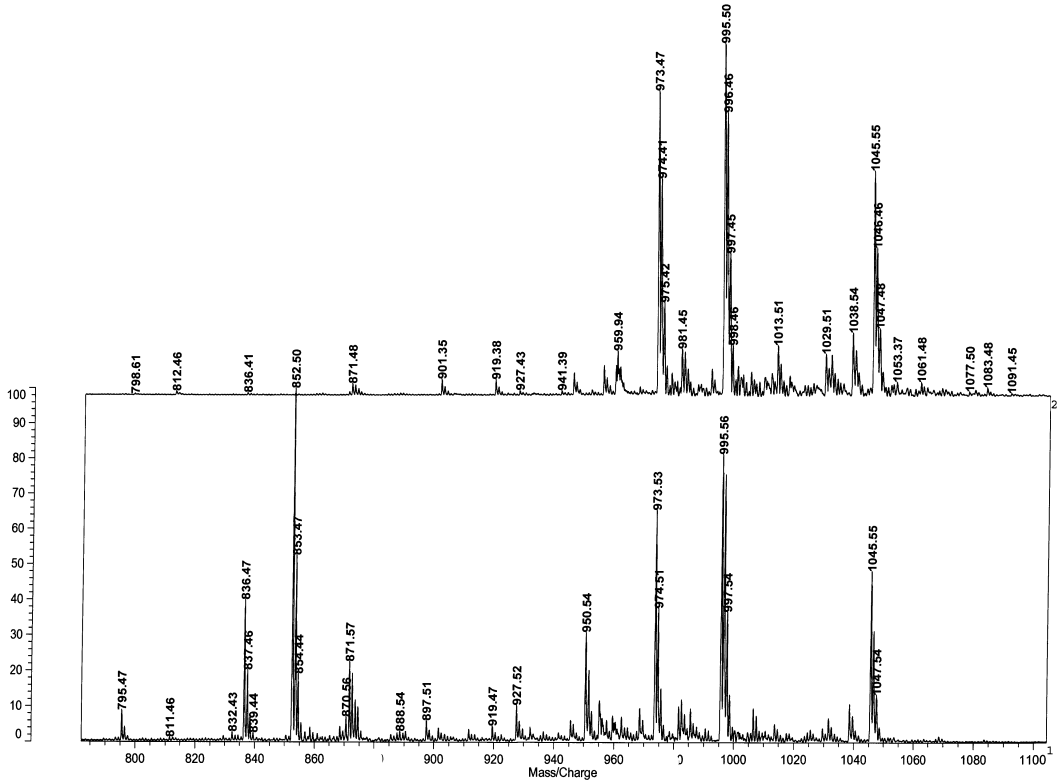


Figure 11. MALDI-TOF mass spectrum of *Microcystis aeruginosa* strain.

375 g of dried *Microcystis aeruginosa* was extracted with dichloromethane/ methanol (1:1) for three times then followed by methanol for three times to afford 50 g crude extract. A lot of chlorophylls, salts and fats were contained in the crude extract The crude extract of the *Microcystis aeruginosa* strain was fractionated by silica gel flash chromatography to afford four fractions.. From fraction I and II, most of the metabolites were isolated as fat. Fraction III and IV was subjected to HPLC/MS. The HPLC/ESI mass spectra of the fraction IV (Figure 12) indicated a mixture of three microcystins, two of which showed *quasi*-molecular ions of $[M + H]^+$ at *m/z* 995 and 1045, respectively. For further purification, fraction IV was given on Sephadex LH-20 followed by silica gel column. By applying the above spectroscopic data to AntiBase, four possible structures were found, two for each mass: microcystin LR (**37**), [D-Asp3,(E)-Dhb7]microcystin-LR (**38**), for the mass 995 and microcystin YR (**39**), [D-Asp3,(Z)-Dhb7]microcystin-HtyR (**40**), for mass of 1045. The structures of these peptides were elucidated by detailed ESI/MS/MS studies, as described below.

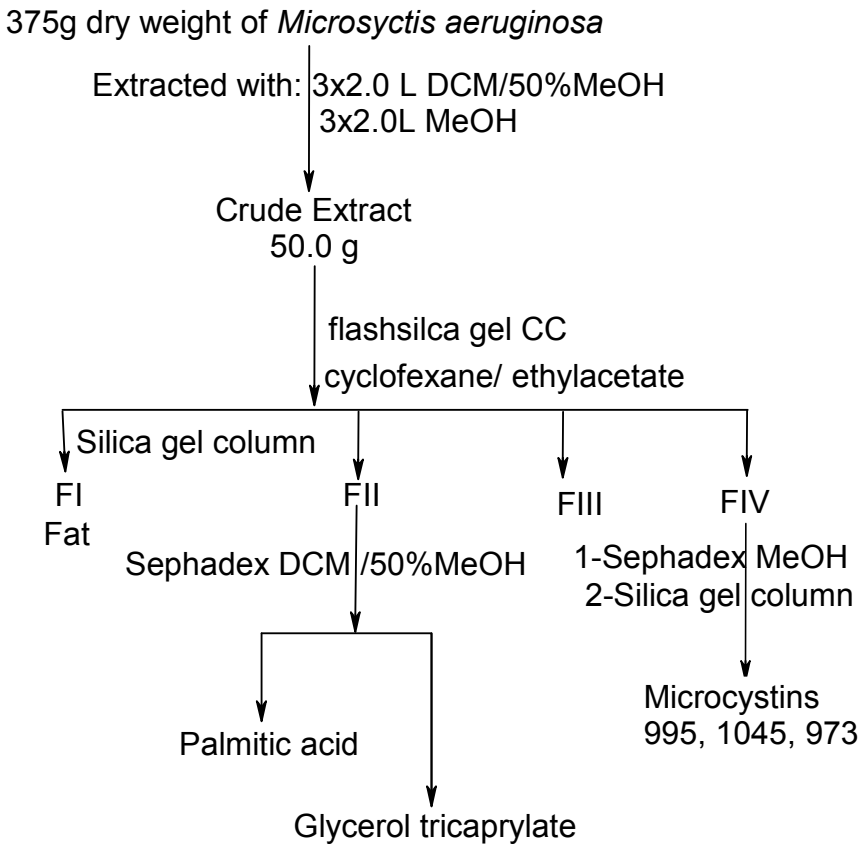
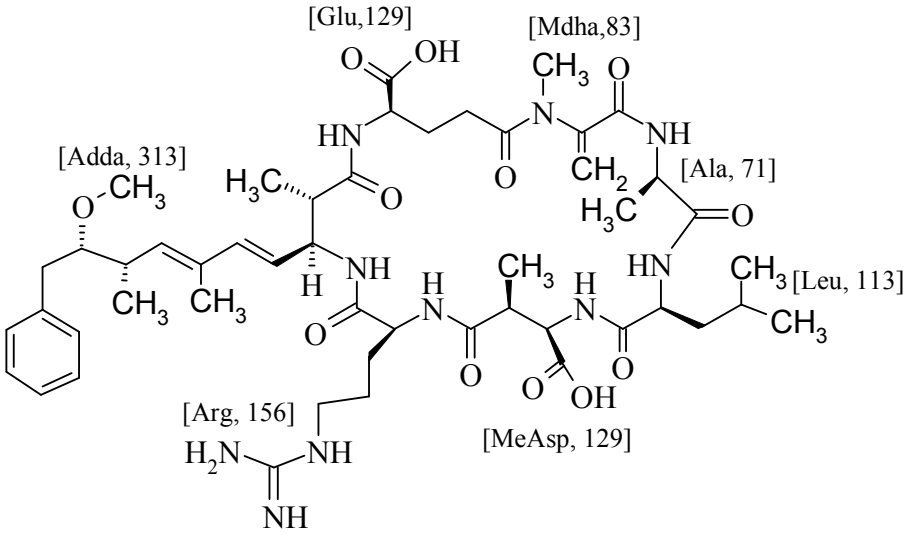


Figure 12. Work up scheme of *Microcystis aeruginosa*.

3.2.1 Microcystin LR

The ESI MS^2 and MS^3 fragmentation of *m/z* 995 $[M + H]^+$ revealed sequential losses of amino acids, confirming the sequence as *cyclo*[Adda-Arg-MeAsp-Leu-Ala-Mdha-Glu+H]$^+$. The intense peak observed at *m/z* 976 in MS^2 confirmed the cleavage of a lactam bond with the loss of CO. Due to the equivalence of each lactam bond, none was preferentially cleaved resulting in overlapping fragments. The loss of Glu (129 amu) was indicated by the peak at *m/z* 866 and the further sequential loss of Mdha (83 amu), Ala-Leu (184 amu) and MeAsp (129 amu) resulted in the fragments at 783, 599 and 470, respectively. The peak at *m/z* 470 was due to the remaining protonated Adda-Arg moiety. Additionally, the peaks observed at *m/z* 683 and 710 were due to the loss of Adda and Arg-MeAsp, respectively, from the parent ion $[M + H]^+$. In most cases the loss of each amino acid fragment was accompanied by the loss of CO and H_2O. The structure of **38** was excluded on the basis of the absence of peaks at *m/z* 715 and 585, which would account for losses of the Arg-Asp and NorLeu-Ala-Mdha-Glu fragments, respectively. Therefore, the structure was determined as microcystin LR (**37**).



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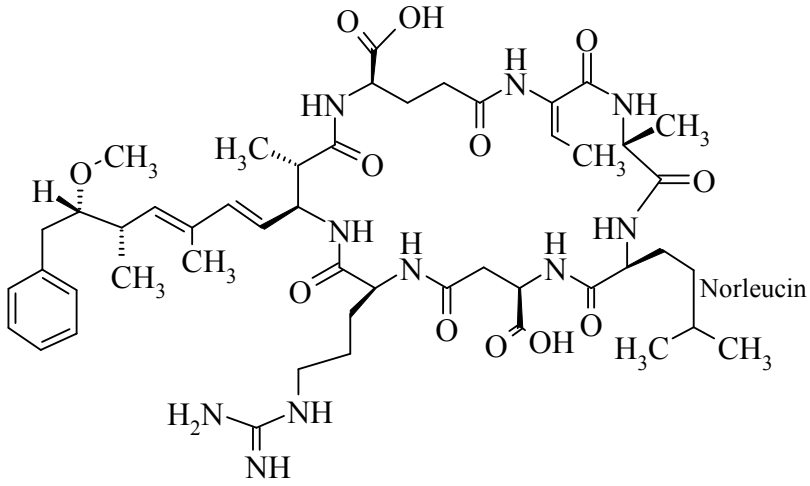

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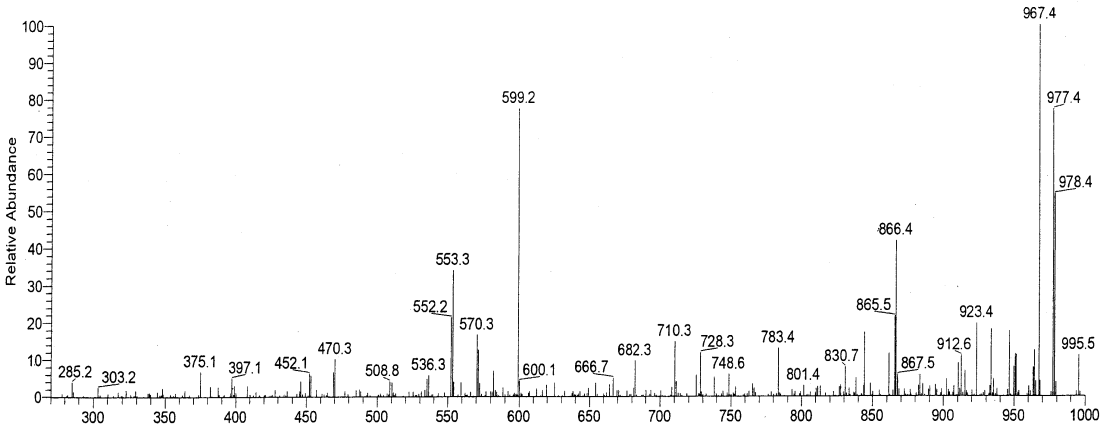


Figure 13. The MS^2 of *m/z* 995.5 $[M + H]^+$ of microcystin LR (**37**).

Table 1. Specific fragment ions of microcystin LR (**37**).

Fragmentation	Δm	Mass	Sequence of amino acid
$[M + H]^+$		995	*Cyclo*[Adda-Arg-MeAsp-Leu-Ala-Mdha-Glu+H]$^+$
$[M+ H–H_2O]^+$	18	977	[Adda-Arg-MeAsp-Leu-Ala-Mdha-Glu+H-H_2O]$^+$
$[M+ H–CO]^+$	28	976	[Adda-Arg-MeAsp-Leu-Ala-Mdha-Glu+H –CO]$^+$
$[M+ H–CO–H_2O]^+$	46	949	[Adda-Arg-MeAsp-Leu-Ala-Mdha-Glu+H –CO–H_2O]$^+$
$[M+ H–Glu]^+$	129	866	[Adda-Arg-MeAsp-Leu-Ala-Mdha+H]$^+$
$[M+H–Glu–CO–H_2O]^+$	46	820	[Adda-Arg-MeAsp-Leu-Ala-Mdha+H–CO–H_2O]$^+$
$[M+H-Glu-Mdha]^+$	212	783	[Adda-Arg-MeAsp-Leu-Ala+H]$^+$
$[M+H–Arg-MeAsp]^+$	285	710	[Adda-Glu-Mdha-Ala-Leu+H]$^+$
$[M+H–Adda]^+$	313	682	[Arg-MeAsp-Leu-Ala-Mdha-Glu+H]$^+$
$[M+H–$ Leu-Ala-Mdha-Glu$]^+$	396	599	[Adda-Arg-MeAsp+H]$^+$
$[M+H–$Leu-Ala-Mdha-Glu–CO–$H_2O]^+$	442	553	[Adda-Arg-MeAsp+H–CO–H_2O]$^+$
$[M+H–$ Adda-Glu$]^+$	442	553	[Arg-MeAsp-Leu-Ala-Mdha+H]$^+$
$[M+H–$ MeAsp-Leu-Ala-Mdha-Glu$]^+$	525	470	[Adda-Arg+H]$^+$
[M– MeAsp-Leu-Ala-Mdha-Glu	526	469	Adda-Arg

Mdha = N-methyl dehydroalanine, Adda = (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid

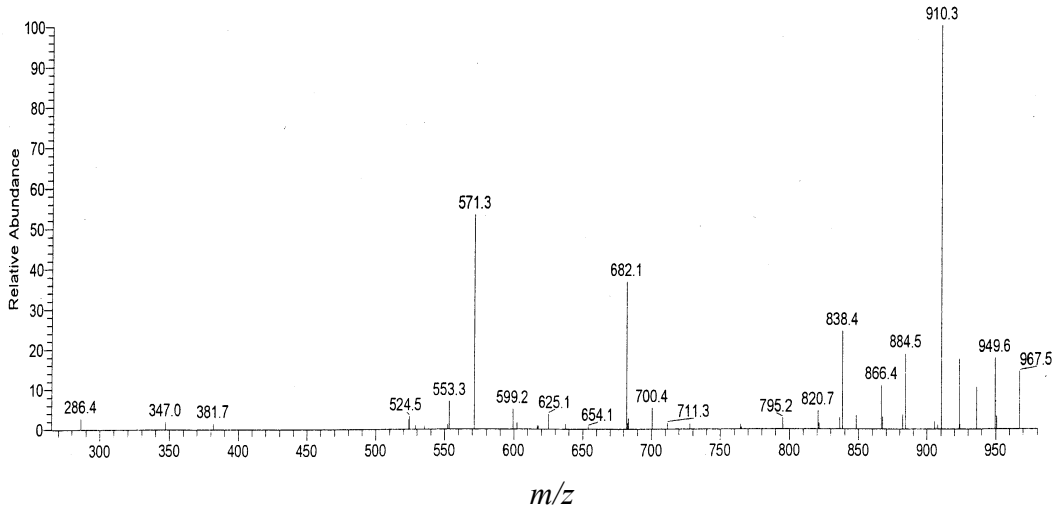
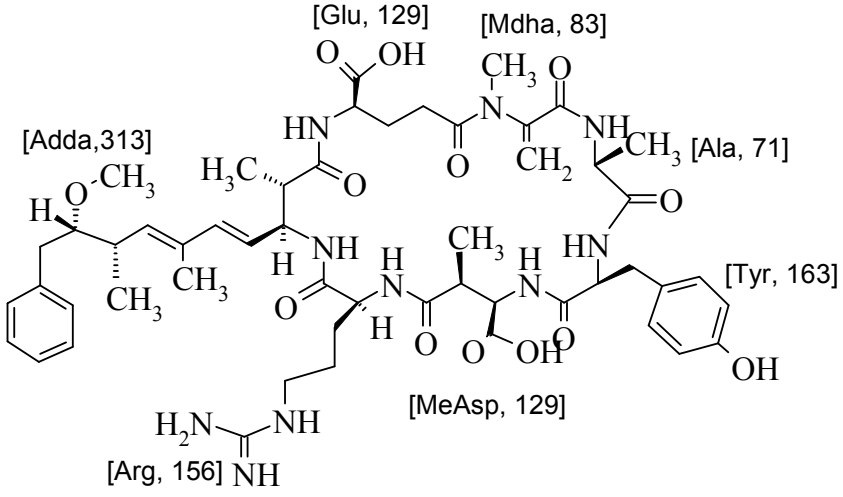


Figure 14. The MS^3 of *m/z* 995.5 $[M + H]^+$ of microcystin LR (**37**).

3.2.2 Microcystin YR

The ESI MS^2 measurement of *m/z* 1045 $[M + H]^+$ resulted in a similar fragmentation pattern as microcystin LR with the peak at *m/z* 599 common to both. The sequential losses of Glu, Mdha, Ala-Tyr and MeAsp were seen from the peaks observed at *m/z* 916, 833, 599 and 470 respectively. This indicated that leucine was replaced by tyrosine resulting in the sequence *cyclo*[Adda-Arg-MeAsp-Tyr-Ala-Mdha-Glu+H]$^+$. The presence of Tyr was confirmed only by mass difference since no fragment ion was observed for the loss of Ala-Mdha-Glu (*m/z* 762). As in the case of microcystin LR, the loss of some amino acid fragments was accompanied by the loss of CO and H_2O. The absence of peaks at *m/z* 715 and 585 for the loss of Arg-Asp and NorTyr-Ala-Mdha-Glu fragments, respectively, excluded the structure of **40** as a possibility.



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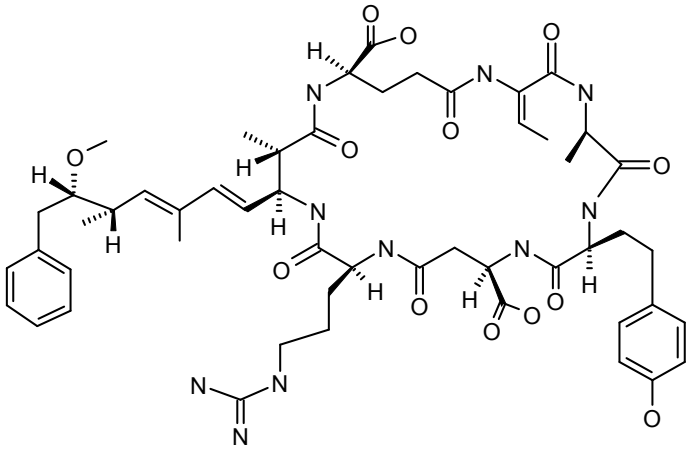

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Table 2. Specific sequence ions of microcystin YR (**39**).

Fragmentation	*Δ*m	Mass	Sequence of amino acid
$[M+H]^+$		1045	*Cyclo*[Adda-Arg-MeAsp-Tyr-Ala-Mdha-Glu+H]$^+$
$[M+H–H_2O]^+$	18	1027	[Adda-Arg-MeAsp-Leu-Ala-Mdha-Glu+H-H_2O]$^+$
$[M+H–CO]^+$	28	1017	[Adda-Arg-MeAsp-Tyr-Ala-Mdha-Glu+H –CO]$^+$
$[M+H–CO–H_2O]^+$	46	1000	[Adda-Arg-MeAsp-Tyr-Ala-Mdha-Glu+H –CO–H_2O]$^+$
$[M+H–Glu]^+$	129	916	[Adda-Arg-MeAsp-Tyr-Ala-Mdha+H]$^+$
[M+H-Glu-Mdha]$^+$	212	833	[Adda-Arg-MeAsp-Tyr-Ala+H]$^+$
[M+H–Tyr-Ala-Mdha-Glu]$^+$	396	599	[Adda-Arg-MeAsp+H]$^+$
[M+H–Adda-Glu]$^+$	442	603	[Arg-MeAsp-Tyr-Ala-Mdha+H]$^+$

Microcystins are a family of monocyclic nonribosomal heptapeptide toxins, produced by several species of fresh water cyanobacteria, namely *Microcystis*. The microcystins consist of D-alanine at position 1, γ-linked D-glutamic acid at position 6, three unusual amino acids, *β*-linked D-*erythro-β* methylaspartic acid (MeAsp) at position 3, (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic-acid (Adda) at position 5, *N*-methyl dehydroalanine (Mdha) at position 7 and two variable L-amino acids at positions 2 and 4. Over 50 different microcystins that differ mainly in the two L-amino acids at positions 2 and 4, and methylation/demethylation on MeAsp and Mdha have been reported. The amino acid Adda plays a major role for the expression of biological activity.[41,42] Among microcystins, the most frequently

found ones are microcystin–LR where the variable L-amino acids are S/L-leucine and R/D-arginine.[41] The Adda and D-glutamic acid of the microcystin-LR molecule are responsible for the hepatotoxicity of microcystins. Methylation of the free carboxyl group of glutamic acid results in the production of inactive compounds.[43] The two hepatotoxic cyclic heptapeptide microcystin LR (**37**), microcystin YR (**39**) were isolated from *Microcystis aeruginosa*[44] and *Nostoc* strains.[45]

3.2.3 Compound 973

HRMS delivered a mass of 973.53557 $[M + H]^+$ corresponding to the molecular formula $C_{46}H_{72}N_{10}O_{13}$ and to sixteen double bond equivalents. HPLC-ESI-MS/MS revealed four major fragments at *m/z* 955 (due to the loss of water), 760, 386 and 150. The molecular formula for the fragment at *m/z* 760 was deduced as $C_{36}H_{57}N_9O_9$ after the loss of $C_{10}H_{15}NO_4$. The fragment at *m/z* 760 was accompanied by the sequential loss of two water molecules at *m/z* 742 and 724. HRMS for the fragment at *m/z* 386 and 150 were $C_{21}H_{27}N_3O_4$ and $C_9H_{11}NO$, respectively. The above three molecular formulae ($C_{10}H_{15}NO_4$, $C_9H_{11}NO$) did not correspond to any amino acid in a microcystin, and also not to a dipeptide, as only one nitrogen atom is present. The molecular formula of $C_{21}H_{27}N_3O_4$ could tentatively assigned to (Ahp-Leu-MTyr)-H_2O with Ahp = 3-Amino-6-hydroxy-piperidone, but there are no further proofs.

Microcystins are delivering a characteristic fragment ion derived from Adda at *m/z* 135, which is generated by in-source collision induced dissociation (in-source CID).[46] Both Microcystin LR (**37**)[47,48] and microcystin YR (**39**)[49] showed this fragment ion (*m/z* 135), according to the literature. However, the fragmentation of compound 973 using ESI-MS/CID did not deliver this characteristic fragment ion peak, so that a new microcystin could not be confirmed for sure. One the other hand, the *m/z* 135 ion was also in the spectrum of **37** not visible, perhaps due to insufficient concentration.

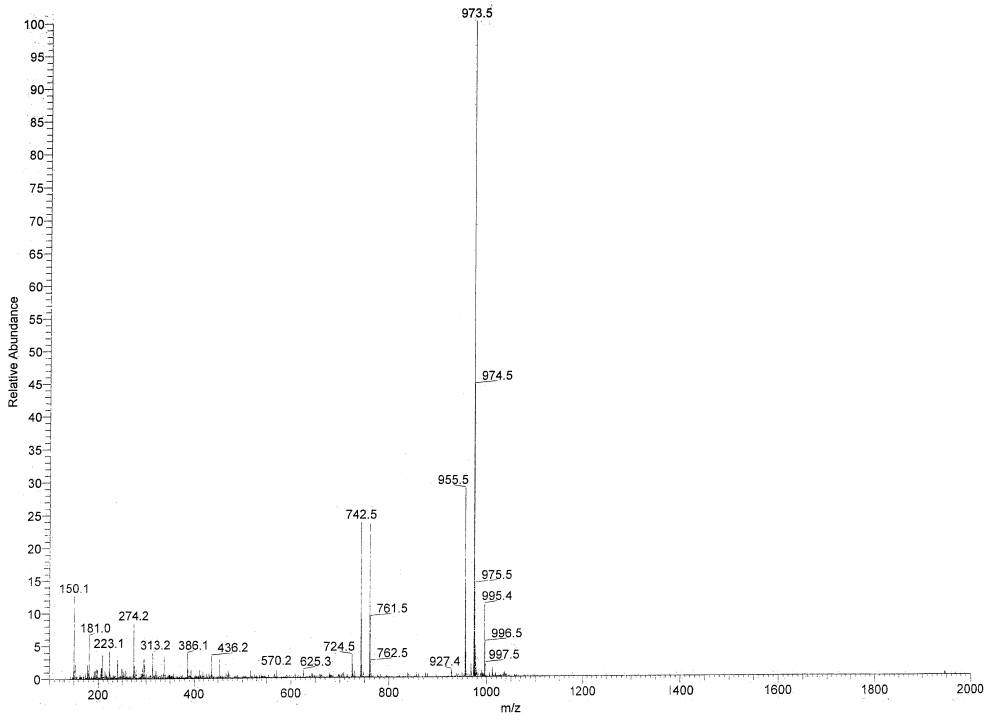
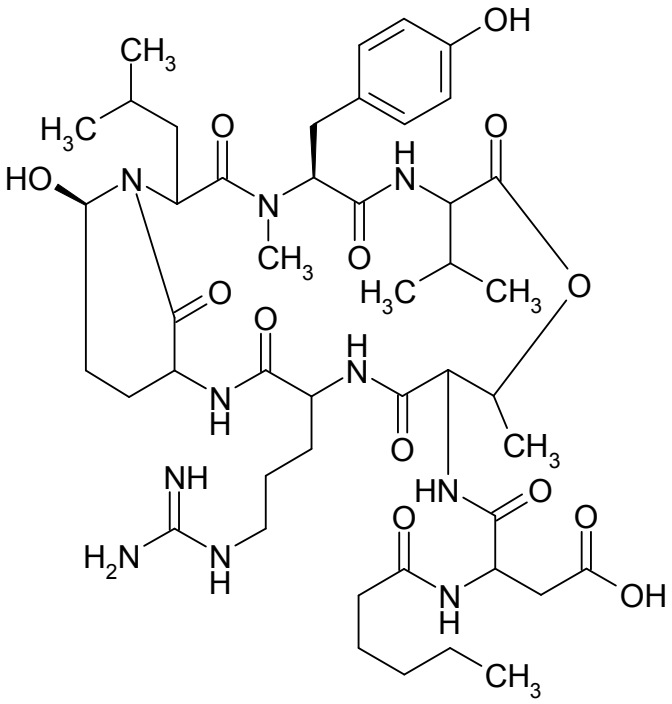


Figure 15. ESIMS/CID mass spectrum of compound (*m/z* = 973).

The ^{1}H NMR spectrum of fraction 973 showed signals in the aromatic region, in the region of α-protons in peptides between δ 3.5~4.5. The aliphatic signals at 2.3, 1.6, 0.8, and the strong methylene signal at 1.3 indicated a long aliphatic chain, which is not present in any of the known microcystins. Although the spectrum was badly resolved, no signal was visible in the olefinic region between δ 5~6.5. The reaction with ninhydrin was negative. These data are better agreeing with the structure of a cyanopeptolin than of a microcystin: The best agreement was found with cyanopeptolin 972 (**41**),[50,51] which has the same empirical formula and lets us expect also spectroscopic characteristics, which should be similar to the observed values. A final proof will need, however, further measurements and more material.



41

3.3 Anabaena constricta

Research on cyanobacteria metabolites includes both studies to discover new potential pharmaceuticals or natural toxins and studies to enhance knowledge of the nature and metabolism of cyanobacteria. In this regard, filamentous cyanobacteria, e.g. species of the genus *Anabaena*, turned out to be a particularly valuable source of potent biotoxins, such as the well-investigated neurotoxic alkaloids anatoxin-a and anatoxin-a(s).[52] As a result of previous screening of several cyanobacteria and microalgae for the presence of antimicrobial (anticyanobacterial) extracellular metabolites,[53] bioactive culture medium extracts of the cyanobacterium *Anabaena constricta* were investigated in detail. The antimicrobial constituent was identified as a brominated indol-2-one derivative, designated as bromoanaindolone.

3.3.1 Bromoanaindolone

The antimicrobial extracts of culture media of the cyanobacterium *Anabaena constricta* were found to be of a conspicuously simple composition, with only one dominating constituent Figure 16. This compound, named bromoanaindolone, was purified *via* preparative TLC, and obtained as a colourless powder.[54]

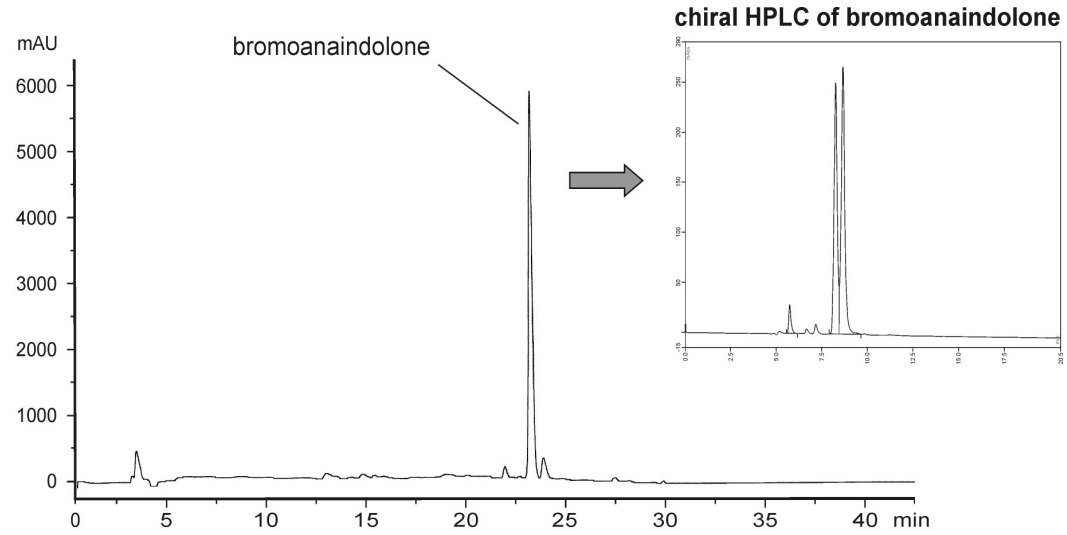


Figure 16. HPLC of a typical culture medium extract of *Anabaena constricta*.

The IR spectrum of bromoanaindolone showed a strong band at 1734 cm^{-1}, suggesting the presence of a carbonyl group. The molecular formula was determined by EIMS and ESIHRMS as $C_9H_8BrNO_2$, which was in accordance with NMR experiments. The ^{1}H NMR spectrum contained signals for three aromatic protons, at δ 7.23 (d, 1H, ^3J = 7.9 Hz), δ 7.14 (dd, 1H, ^3J = 7.9, 1.7 Hz) and δ 6.95 (d,1H, ^4J = 1.7 Hz), which pointed to the presence of a trisubstituted aromatic ring with substituents in the 1,2 and 4-positions. In addition, the ^{1}H NMR spectrum showed signals for five other protons, at δ 10.32 (s, 1H), δ 5.92 (s, 1H) and δ 1.35 (s, 3H).

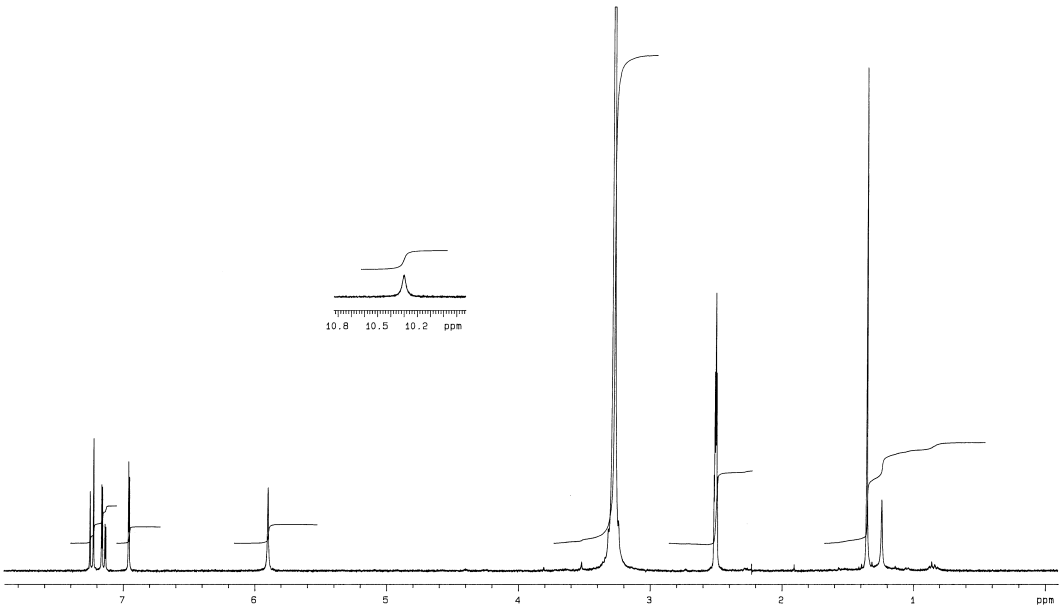


Figure 17. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of bromoanaindolone (**42**).

The ^{13}C NMR spectrum contained signals for six aromatic carbon atoms at δ 142.8 (C_q-7a), 132.8 (C_q-3a), 125.2 (C-4), 124.1 (C-5), 121.2 (C-6), 112.3 (C_q-6) respectively. In addition, a carbonyl group at δ 179.3 (C-2), an oxygenated quaternary carbon atom at δ 72.2 (C-3), and a methyl group at δ 24.1 (C-8) were visible. Further assignments were obtained from the HSQC and DEPT 135 spectra. As no ^{1}H or ^{13}C correlations were found for both 1H singlets at δ 10.32 and at δ 5.92, it was assumed that these protons were attached to heteronuclei rather than to carbon. Using the molecular formula $C_9H_8BrNO_2$, and also the IR data, the presence of a hydroxyl group and a secondary amine was therefore proposed. Based on these findings, the basic structure of the molecule was determined to be that of an indolone or an iso-indolone alkaloid (Figure 21).

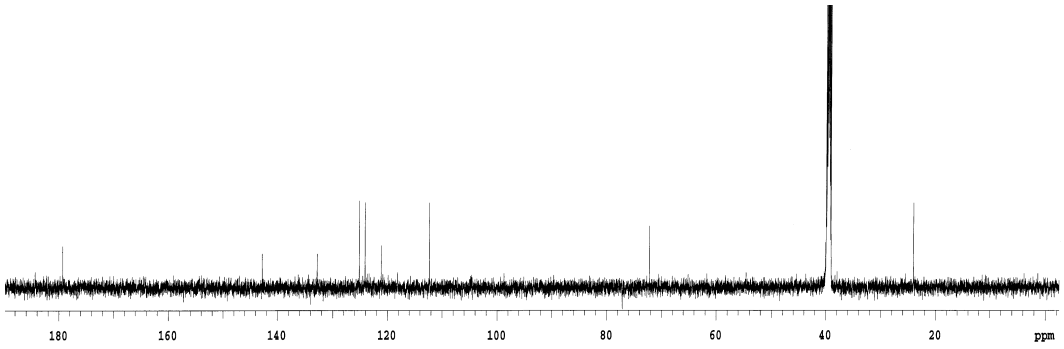


Figure 18. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of bromoanaindolone (**42**).

In the homonuclear H,H COSY spectrum, the aromatic proton signal at δ 7.14 (H-5) was *o*-coupled with H-4 at δ 7.23 and *m*-coupled with H-7 at δ 6.95 which confirmed the presence of a 1,2,4-trisubstituted aromatic ring. The ^{3}J HMBC correlation of the aromatic proton H-4 (δ 7.23) to the oxygenated quaternary carbon C-3 (δ 72.2) and the aromatic quaternary carbon C-6 (δ 121.2) located C-3 and Br at *ortho* and *meta*-position to H-4 respectively, which also excluded the possible structure of an indol-3-one. The aromatic proton H-7 (6.95) did not show any correlation to the carbonyl at 179.3, which in combination with the upfield shift of H-7 indicated, that this proton was in an *ortho*-position of an electron donating group which; this also excluded the isoindolone alternative. Finally the HMBC correlations listed in Table 3 were measured.

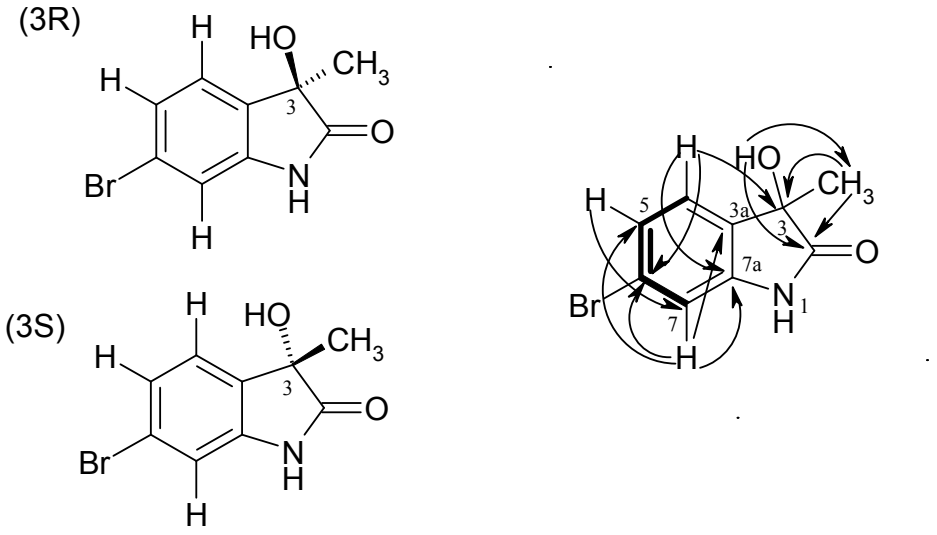


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Figure 19. Structure (left), HMBC (→) and H,H COSY (▬) correlations (right) of bromoanaindolone (**42**).

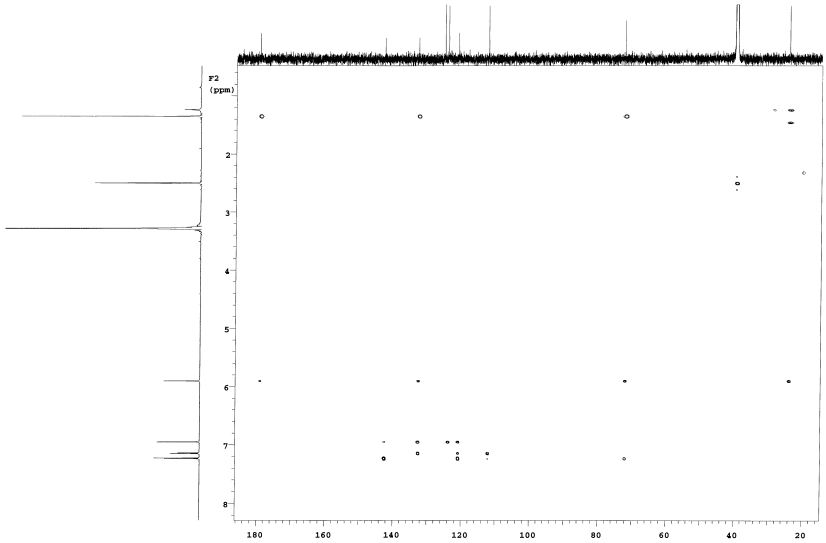
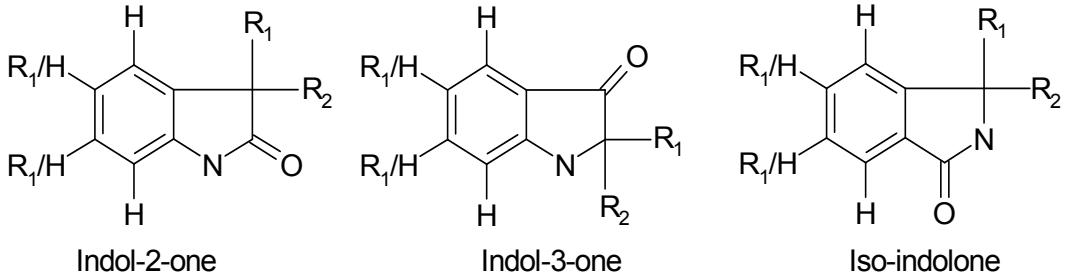


Figure 20. HMBC spectrum (DMSO-d_6, 150 MHz) of Bromoanaindolone (**42**).



$R_{1/2/3}$ = -Br / -OH / -CH_3

Figure 21. Possible structures of bromoanaindolone.

Table 3. NMR data (^{1}H 300 MHz, ^{13}C 75 MHz) of bromoanaindolone (**42**).

position	δ_H	J (Hz)	δ_C	HMBC[a]
1	10.32	br s		
2			179.3	
3			72.2	
3a			132.8	
4	7.23	d, 7.9 (H-5)	125.2	C-3, C-6, C-7a
5	7.14	dd, 7.9 (H-4), 1.7 (H-7)	124.1	C-3a, C-6, C-7
6			121.2	
7	6.95	d, 1.7 (H-5)	112.3	C-3a, C-5, C-6, C-7a
7a			142.8	
8	1.35	s	24.1	C-2, C-3, C-3a
3-OH	5.92	br s		C-2, C-3, C-3a, C-8

[a] Denotes HMBC correlations from proton at position X to the corresponding carbon.

Bromoanaindolone (**42**) showed a negative CD signal at 238 nm and a positive one at 268 nm, pointing to a (3*R*) configuration. All related natural 3-hydroxyoxindoles with a (3*R*) configuration demonstrate similar behaviour, being reported to exhibit at least two Cotton effects in the UV range, with a negative signal at 225-250 nm and a positive one at 260-300 nm.[55-57] The optical rotation was, however, close to zero, indicating an enantiomeric mixture with a slight excess of the (3*R*) enantiomer. This was subsequently found to be the case, as analytical HPLC on a chiral column of the pure indolone, as well as of crude culture medium extracts, afforded two well-separated signals of nearly identical intensity (Figure 16).

Using the microdilution method for determination of the minimum inhibitory concentration (MIC), bromoanaindolone (**42**) showed antibacterial activity against the Gram-positive bacterium *Bacillus cereus* (MIC 128 µg ml^{-1}). In a special solid matrix assay for determination of the minimum toxic quantity (MTQ), bromoanaindolone displayed anticyanobacterial activity against the filamentous species *Arthrospira laxissima* (MTQ 18 µg) and *Nostoc carneum* (MTQ 20 µg), and against the unicellular species *Chroococcus minutus* (MTQ 20 µg), *Synechocystis aquatilis* (MTQ 16 µg) and *Synechococcus* sp. (MTQ 8 µg).

Indole alkaloids are widely distributed in the plant kingdom, and closely related oxindoles have previously been isolated from streptomycetes[58] or marine bryo-

zoa.[59,60] For example, 4,6-dibromoanaindolone (convolutamydine C) has been isolated from the marine bryozoan *Amathia convolute*.[59] It can be speculated that such compounds are of cyanobacterial origin. There are, however, only a few reports of halogenated indole alkaloids isolated from cyanobacteria. These include a group of poly-brominated biindoles from *Rivularia firma*,[61,62] three chlorinated carboline derivates (designated as bauerines) from *Dichotrix baueriana*[63] and a chlorinated quaternary carboline alkaloid (designated as nostocarboline) from *Nostoc* 78-12A.[64] The natural function of the newly discovered exometabolite of *Anabaena constricta* has not been established to date. Speculatively, however, bromoanaindolone may act as an allelopathic substance, and may help *A. constricta* with the combat of competing microorganisms in the same habitat, as has previously been proposed for other antimicrobial exometabolites of cyanobacteria.[65]

4 General Techniques for Streptomycetes

4.1 Collection of strains

All marine derived *Streptomyces* sp. (B-strains) were isolated and taxonomically identified by E. Helmke, Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven. Terrestrial *Streptomycetes* spp. with the strain label 'Ank' investigated here were isolated from soil samples in 2006 and identified by H. Anke, IBWF. The terrestrial *Streptomycetes* with the code 'GT' were isolated in the Hans-Knöll-Institute from soil samples received from University Yunnan, China. The terrestrial Wo strains belong to a project with Prof. Wolf in a cooperation with the BASF AG on the search for compounds with activity against plant-pathogenic fungi.

4.2 Biological and chemical screening

To select the strains with biological activities and interesting properties from the received strains, a so-called pre-screening for biological and chemical properties is performed. The strains are sub-cultured on agar plates for 3-7 days and microscopically examined for contaminations. Well-grown agar sub-cultures were cut into small pieces to inoculate 1 L Erlenmeyer flasks containing 250 mL of medium. The culture was cultivated on a rotary shaker at 28 °C. The culture broth was then lyophilised and the dried residue was exhaustively extracted with water-saturated ethyl acetate. The obtained crude extract was used for biological, chemical and pharmacological screenings and also for HPLC MS. In biological screening, the extracts were subjected to agar diffusion tests using *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis, Staphylococcus aureus*, the fungi *Mucor miehei* (Tü284) and *Candida albicans,* and the micro-algae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus* as test organisms. In parallel, the cytotoxic activity was evaluated against brine shrimps (*Artemia salina*). In the chemical screening, the extracts were subjected to TLC and checked by UV and spray reagents as well as by HPLC analysis to avoid the replication of known compounds. The following spray reagents were used in our group:

Anisaldehyde/sulphuric acid: gives various colour reactions with many structurally diverse compounds.

Ehrlich's reagent shows colour reactions with indole derivatives and some heterocycles. Indoles stain to pink or violet, pyrroles and furans show brown colouration, anthranilic acid derivatives change to yellow.

Concentrated sulphuric acid: principally used for polyenes which turn to brown or black; a blue colour indicates a carotenoid, green is pointing to a carotenoid containing carbonyl in conjugation with the double bonds. The similarly coloured actinomycins are changing to a darker orange colour.

Sodium hydroxide: used for revealing *peri*-hydroxy-quinones, which develop a red, blue or violet colour.

Chlorine/o-dianisidine: used as general reagent for the detection of peptides.

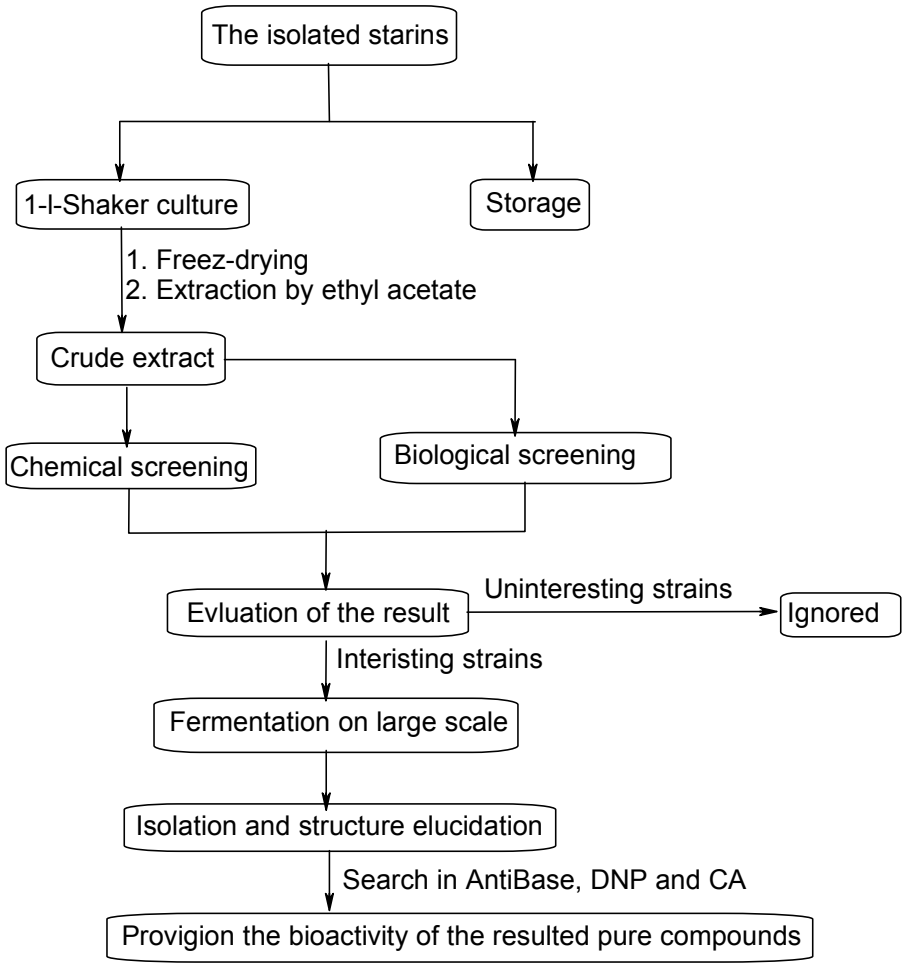


Figure 22. Work up scheme of the selected stains in a general screening.

4.3 Isolation methods

The separation methods were guided according to the amount of the crude extract and the polarity of the compounds of interest. Firstly, the crude extracts were subjected to a column of silica gel eluted with a stepwise gradient of dichloromethane/methanol or cyclohexane/ethyl acetate. Since silica gel is acidic, some compounds may be rearranged, oxidised, cleaved or destroyed by the support. The sepa-

rated fractions from the first column chromatography were further separated by size exclusion column chromatography on Sephadex LH-20, which offers a high recovery rate and minimizes the destruction of compounds. The obtained fractions are monitored by TLC to determine the next isolation steps which may be by PTLC, silica gel column chromatography, Sephadex LH-20, RP-18 or HPLC, etc.

4.4 Cultivation and scale-up

According to the pre-screening, the interesting strains were scaled up for further investigation. In some cases, in order to improve the microbial production of the interesting compounds, the optimisation of the culture conditions may be done. The well-grown agar sub cultures were used to inoculate 100 of 1L Erlenmeyer flasks each containing 250 mL medium and then cultivated on a linear shaker at 28 °C. After some days, the strains were harvested and then mixed with Celite and pressed over filter to separate water phase and biomass. The biomass was exhaustively extracted with ethyl acetate and acetone. The water phase was adsorbed on XAD-16 by passing the solution through a resin column, washed with water and then extracted with methanol. The solvent was evaporated to dryness under vacuum and the remaining crude extract was submitted for further investigation.

4.5 Dereplication

One of the major challenges of the natural product chemist is the discovery and identification of novel and biologically active compounds while reducing the time spent on the isolation of known compounds. Dereplication, the rapid identification of known compounds, is therefore essential. This allows the recognition of known compounds even from a mixture by comparing data fragments.

A number of methods have been developed for this purpose. These include the comparison of UV or mass data in conjunction with HPLC retention times with references from data collections. The advantage of this method is the negligible amount of samples required for analysis and there is no need for purification. Also for unknown metabolites, comparison can be made with similar chromophors and fragmentation patterns of structural analogues. Presently a HPLC-UV-ESI-MS/MS database with over 600 compounds has been developed in our group for the analysis of large numbers of crude extracts. This provides a search format, which requires only retention time, UV and molecular weight information by LC/MS analysis. The result is presented as the MS/MS spectra of monomer and - if available - dimer *pseudo*molecular ions as well as the corresponding UV spectra.

All identifications are finally based on data collections such as the Chemical Abstracts, the Dictionary of Natural Products (DNP) and AntiBase,[26] in which substructures derived from NMR spectra can be searched. AntiBase is best used for the dereplication of compounds from microbial sources. This offers a comprehensive and fast identification of known compounds using a combination of structure fragments, high resolution mass, ^{13}C shifts and other data. Presently there are over 35,000 compounds of many classes in the database and so related structures of new compounds can also be compared. The final confirmation of a compound's novelty is usually achieved by a search in the Chemical Abstracts, which is the most comprehensive collection of compounds worldwide.

5 Marine-derived *Streptomyces* spp.

5.1 Marine-derived *Streptomyces* sp. B7801

The marine strain B7801 was selected due to the physical, chemical and biological characteristics of the crude extract observed in the pre-screening: It was highly active against *Staphylococcus aureus, Mucor miehei* and *Streptomyces viridochromogenes* (Tü 57) and from the TLC, several UV active zones were detected. After spraying with anisaldehyde/sulphuric acid, some of the UV active zones showed an unusual dark green colour along with one of orange colour. From HPLC-MS it was obvious that some metabolites contained chlorine, which is rare in marine *Streptomycetes* metabolites. All these chemical and biological data influenced the decision to work with this strain.

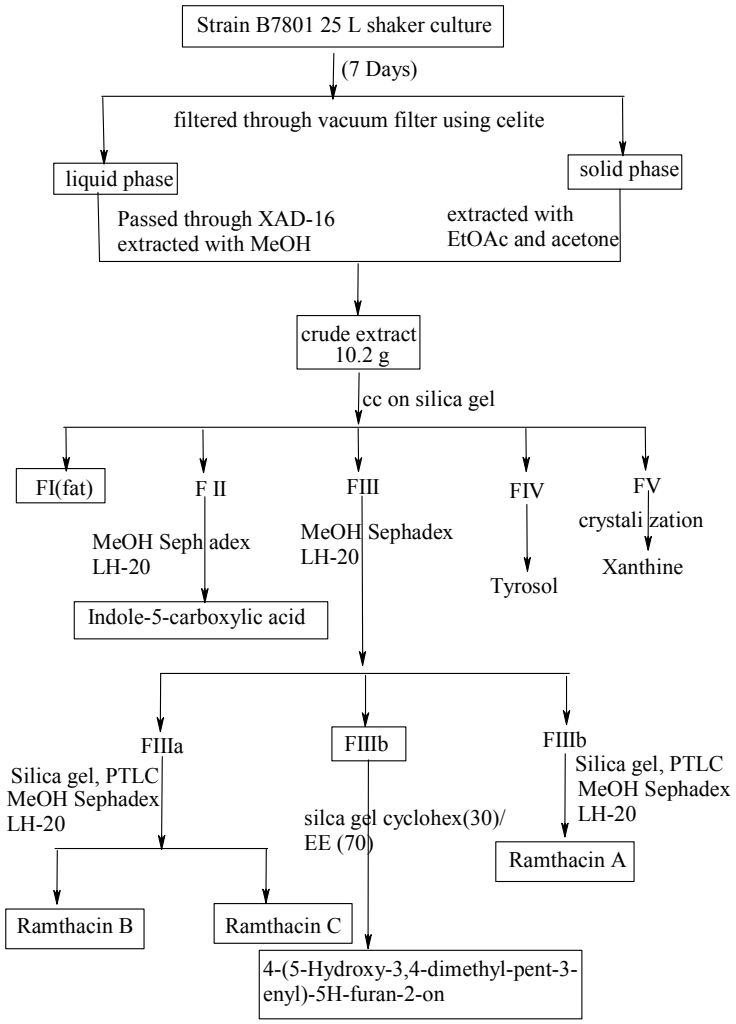


Figure 23. Work-up scheme of the marine *Streptomyces* sp. B7801.

5.1.1 Indole-5-carboxylic acid

The crude extract was fractionated on silica gel column to afford fractions I-V. Compound **43** was isolated from fraction II (Figure 1) as orange solid, by applying it to Sephadex LH-20 with methanol. It was UV-absorbing and gave orange colour reaction with anisaldehyde/sulphuric acid after heating. The ^{1}H NMR spectrum of **43** displayed five 1H signals in the aromatic region. The proton at δ 7.80 showed *o*-coupling with that at δ 7.41 and *m*-coupling with δ 8.30. The coupling pattern and the coupling constants indicated a 1,2,4-trisubstituted benzene ring. Two further protons were observed as two doublets at δ 7.32 and 6.65 with a small coupling constant of 3.7 Hz. This is the typical value for five-membered heterocyclic rings like pyrrole or furan. EIMS showed the molecular ion peak at *m/z* 161, and HREIMS gave the molecular formula $C_9H_7NO_2$ with seven double bond equivalents in the molecule.

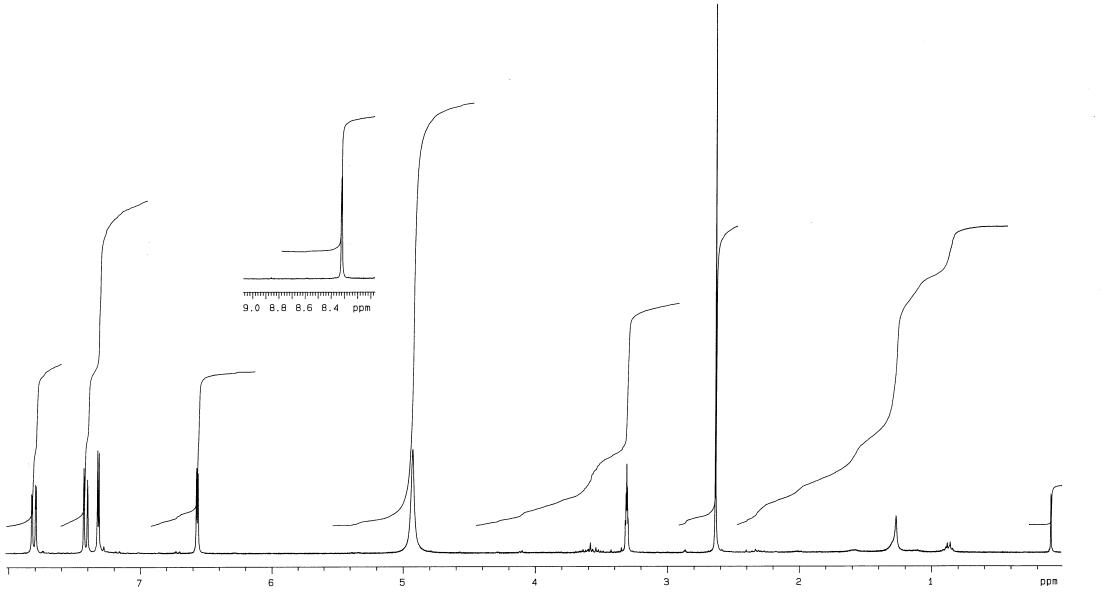


Figure 24. ^{1}H NMR spectrum (CD_3OD, 300 MHz) of indole-5-carboxylic acid (**43**).

The ^{13}C NMR spectrum delivered 9 signals as expected for the molecular formula. There was one carbonyl at δ 171.7 for an acid, ester or amide, five sp^2 methine carbons and three quaternary sp^2 carbons. Due to the shift, the quaternary carbon at δ 140.4 was obviously connected with a hetero atom. The H,H COSY spectrum showed the presence of two fragments: a 1,2,4-trisubstituted benzene (A) and one 1,2-disubstituted pyrrole ring (B).

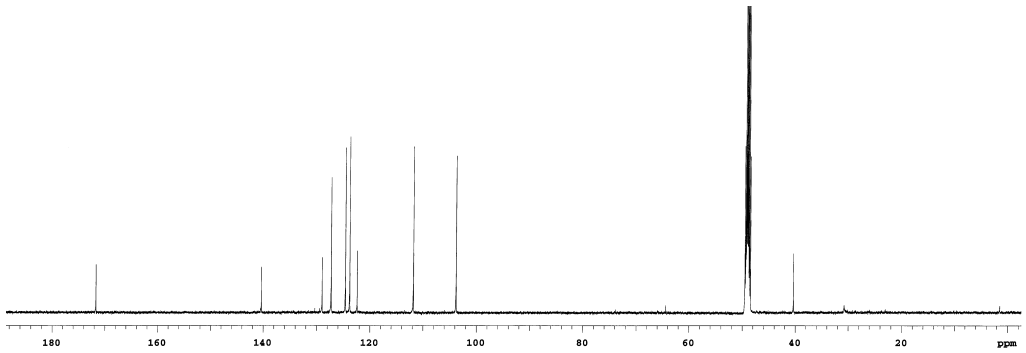


Figure 25. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of indole-5-carboxylic acid (**43**).

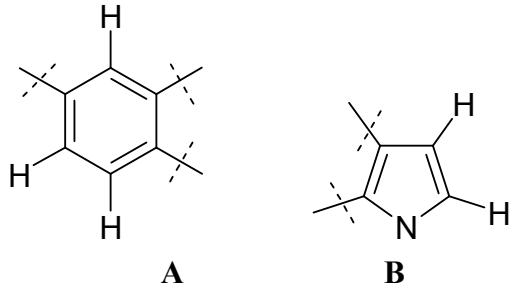


Figure 26. Partial structures of indole-5-carboxylic acid (**43**) from H,H COSY and HSQC spectra.

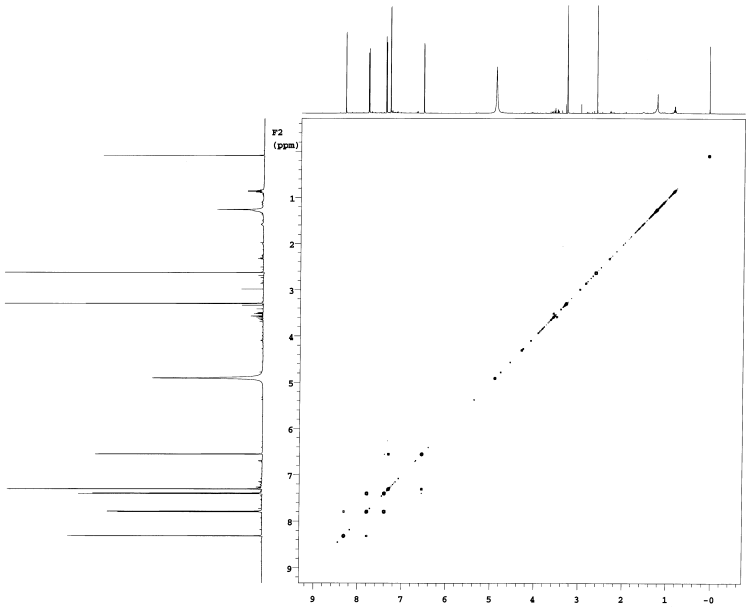


Figure 27. H,H COSY spectrum (CD_3OD, 125 MHz) of indole-5-carboxylic acid (**43**).

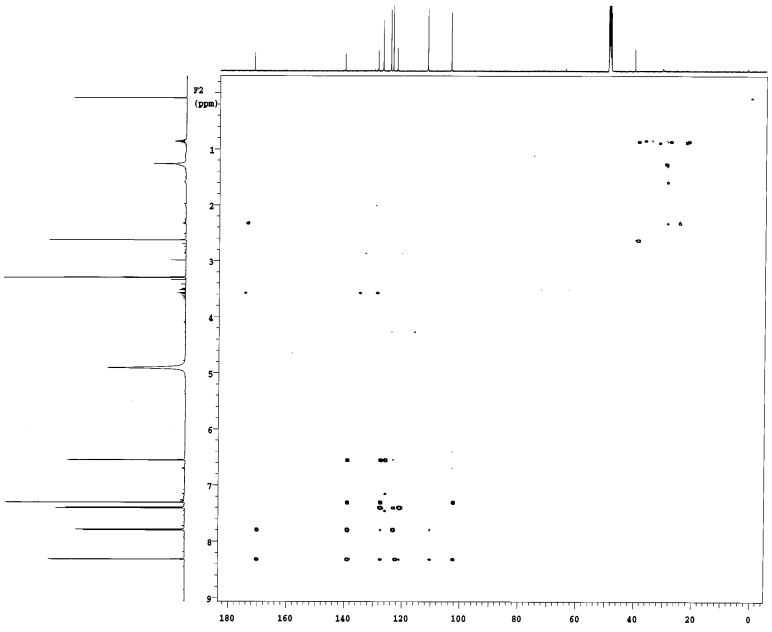
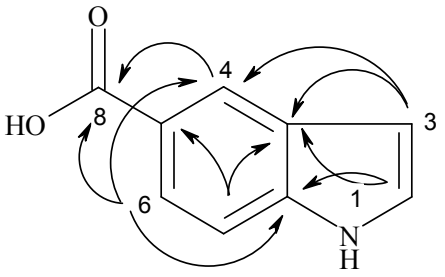


Figure 28. HMBC spectrum (125 MHz, CD_3OD) of indole-5-carboxylic acid (**43**).

A search in AntiBase by using the above spectroscopic data as well as the molecular formula gave six possibilities, among of them the indole-2- and 3-carboxylic acids. These compounds both have an ABCD system for the benzene unit, which was not the case here and also all the other hits did not match with the ^{1}H NMR spectrum, which gave an indication that this compound could be a new natural product from the microorganisms. The compound was finally subjected for 2D NMR measurements, which confirmed the connection of a carboxylic group at C-5 from the ^3J correlation of the *m*-coupled proton at δ 8.30 and also the doublet of doublet at δ 7.80 to the carbonyl at δ 171.7. This confirmed the structure as indole-5-carboxylic acid (**43**).



43

Figure 29. Selective H,H COSY (—) and HMBC (→) connectivities of indole-5-carboxylic acid (**43**).

Indole-5-carboxylic acid was firstly isolated from the root extract of Taichung Native 1 rice in 2001 by Rimando, A. M and coworkers.[66] Plants and microorganisms synthesize the indole moiety from anthranilic acid, which reacts with phosphoribo-

sylpyrophosphate (PRPP) by a S_N2 mechanisms to give N-(5'-phosphoribosyl)-anthranilate. After ring opening of the ribose moiety followed by decarboxylation, indole-3-glycerinphosphate is formed. Methylation of the latter compound is likely to be carried out by S-adenosylmethionine (SAM) followed by reverse aldol reaction to produce indole-5-methyl along with glyceraldehydes-3-phosphate as by-product. Finally, methyl group in indole-5-methyl probably oxidized to indole-5-carboxylic acid.

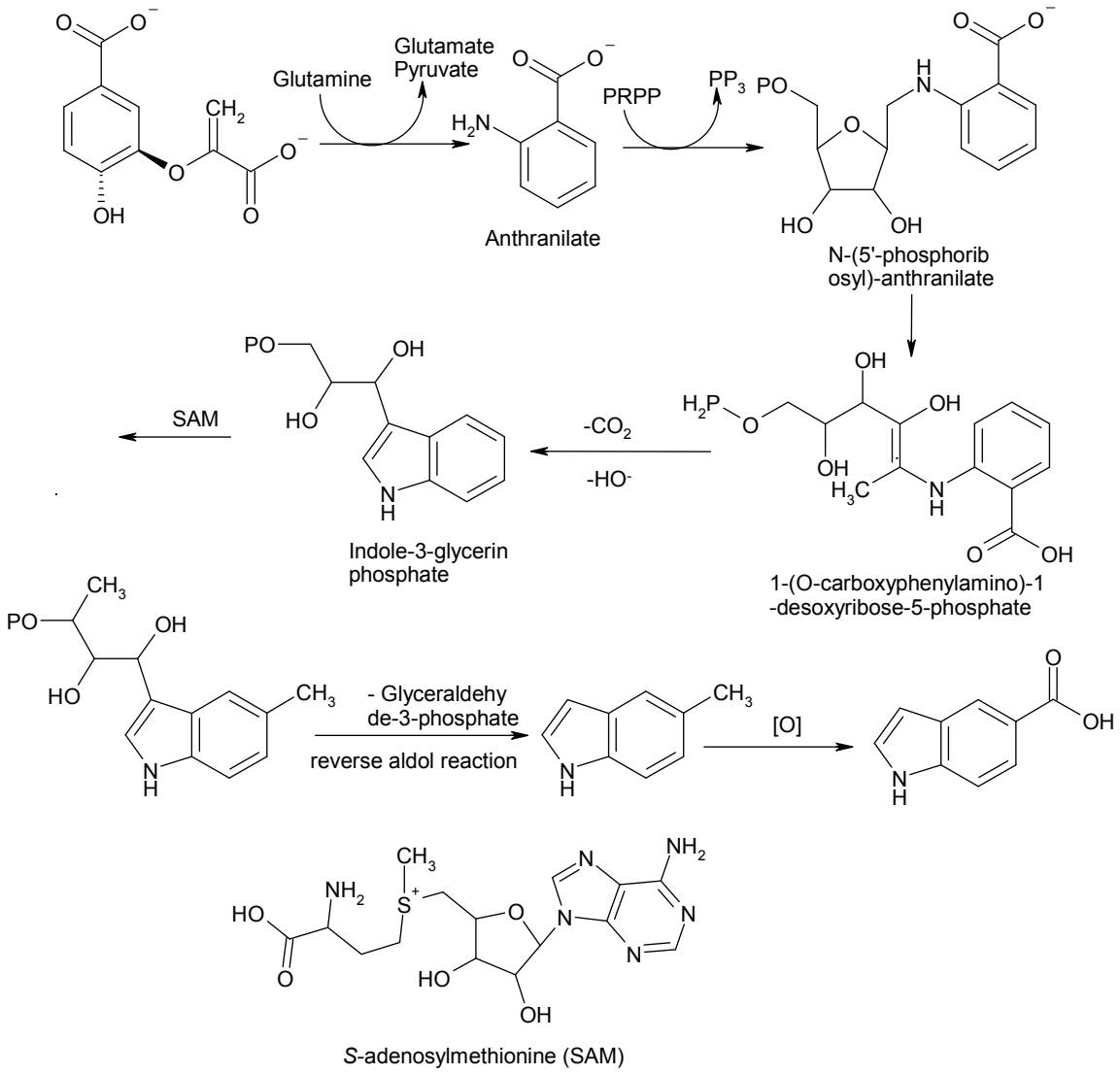


Figure 30. Proposed biosynthetic pathway of indole-5-carboxylic acid (**43**).

Respective 5-methyl-indoles seem to be unknown as natural products, however, the corresponding aldehyde, indole-5-carbaldehyde, has been isolated from *Monodora angolensis*.[67]

5.1.2 Ramthacin A

Compound **44** was obtained as colourless oil with middle polarity from sub-fraction III (Figure 23) by applying to Sephadex LH-20 (MeOH), PTLC (CH_2Cl_2/5% MeOH) and again followed by Sephadex LH-20 (MeOH). It was UV absorbing at 254 nm and stained to dark green colour after spraying the chromatogram with anisaldehyde/sulphuric acid, which changed to brown with time. The molecular weight was established from ESIMS to be *m/z* 349. HRESIMS revealed the molecular formula as $C_{19}H_{24}NO_3Cl$, entailing eight double bond equivalents.

In the ^{1}H NMR spectrum three aromatic protons were observed at δ 7.77 (^3J = 8.3, ^4J = 1.9 Hz), 7.76 (^4J = 1.9 Hz) and 6.52 (^3J = 8.3 Hz). The chemical shift of the signal at δ 6.55 indicated that this proton is in *ortho* position to an electron-donating group like oxygen or nitrogen. From the coupling constants it was clear that two of those protons were *o*-coupled and the third was *m*-coupled as in a 1,2,4-trisubstituted benzene ring. One broad exchangeable proton signal at δ 4.72 emerged which was attributed to OH or NH. In the aliphatic region three methyl groups were observed, two of them were connected to sp^2 carbons an appeared at δ 1.88 and 1.78 as singlets, while the third one appeared at δ 3.40, and could be a methoxy or N-methyl group. At δ 3.36, 3.09, and 2.67, 2.43 two methylene protons were observed as two ABX systems and their down field shift indicated they were in connection with sp^2 carbons or heteroatoms. Another oxygenated methylene singlet was displayed at δ 3.51. One methine triplet at δ 4.23 indicated a neighbouring methylene group and the connection with a heteroatom. In the olefinic region three methine protons were observed: a triplet δ 5.54 (CH-12), and two singlets at δ 5.02 and 4.93(CH_2-15) connected with the same carbon in accordance with the HSQC spectrum. Finally two signals for methyls connected to sp^2 carbons were displayed at δ 1.78 and 1.88.

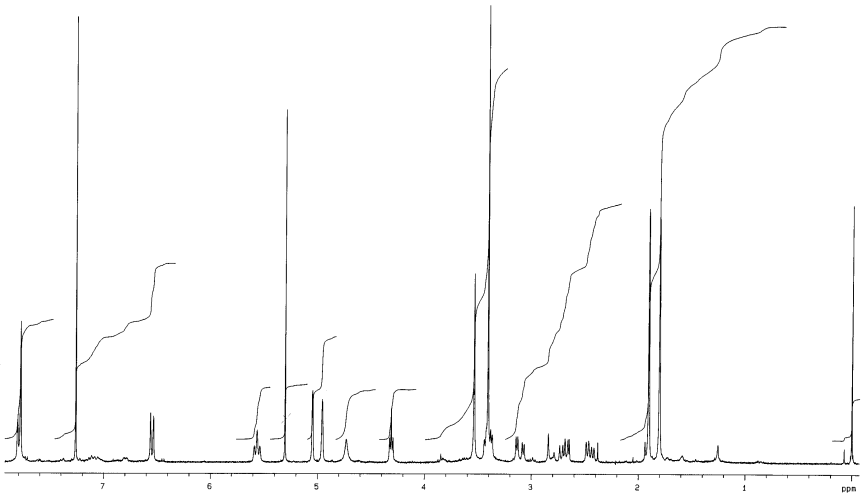


Figure 31. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of ramthacin A (**44**).

The ^{13}C NMR spectrum was in agreement with the molecular formula of **44** and displayed 19 carbon signals which could be categorised as: one carbonyl at δ 171.5 belonging to an acid, ester or amide, 10 sp^2 carbon signals, one oxy-methylene and one oxy-methyl at δ 74.1 and 59.3, respectively. Additionally, one quaternary carbon at δ 58.8 and a methine at δ 56.4 could be attached to an O/N hetero atom, one methylene appeared at δ 33.5 and two methyl groups gave signals at δ 21.0 and 13.9.

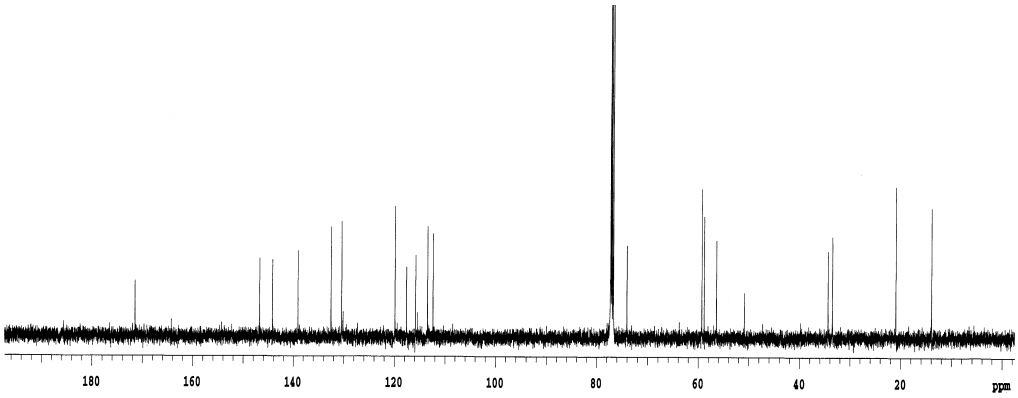


Figure 32. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of ramthacin A (**44**).

A search in AntiBase using the MS and NMR data was without result indicating a new microbial secondary metabolite. Therefore, structure of **44** was subjected to 2D NMR measurements (HMBC and H,H COSY). The COSY spectrum reflected the aromatic protons in 1,2,4-position of the benzene ring, and also the ABX system of the CH-CH_2 fragment (C-3,4) was confirmed. In addition, the methylene protons at δ 2.67, 2.43 (C-11) coupled with the olefinic proton at δ 5.54 (C-12), indicating an allyl position. Finally, the H,H COSY spectrum showed three bond correlations between the ABX methylene group CH_2-4 and the methine proton H-3 (Figure 33).

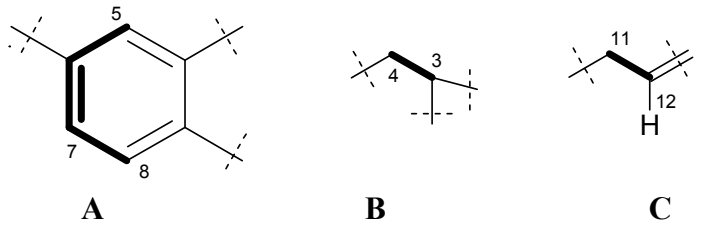


Figure 33. H,H COSY (—) correlations of ramthacin A (**44**).

Table 4. The ^{1}H NMR (300 MHz, $CHCl_3$) data of ramthacin A (**44**), B (**47**), C (**48**).

Position	Ramthacin A(**44**) ^{1}H (mult, *J* [Hz];Int.,)	Ramthacin B (**47**) ^{1}H (mult, *J* [Hz];Int.,)	Ramthacin C (**48**) ^{1}H (mult, *J* [Hz];Int.,)
3	4.23 (t, 4.8 Hz, 1H)	4.60 (t, 11.1,1H)	3.66 (t, 6.6,1H)
OCH_3-3	-	-	3.42 (s, 3H)
4a	3.36 (dd, 16.9, 4.8, 1H)	3.48 (dd,17.5, 4.9, 1H)	3.12 (dd, 17.0, 5.1, 1H)
4b	3.10 (dd, 17.2, 5.1 , 1H)	3.11 (dd, 17.3, 6.2, 1H)	2.85 (dd, 16.7, 6.7, 1H)
5	7.76 (d, 1.9, 1H)	7.77 (d, 1.8, 1H)	7.74 (d,1.8, 1H)
7	7.77 (dd, 8.3, 1.9, 1H)	7.79 (dd, 9.1, 1.8, 1H)	7.78 (dd,8.3, 1.8 , 1H)
8	6.52 (d, 8.3,1H)	6.59 (d, 9.1,1H)	6.57 (d, 8.3,1H)
11a	2.67 (dd, 14.8, 8.5, 1H)	2.05 (m, 1H)	1.97 (dd, 14.8, 10.0, 1H)
11b	2.43 (dd, 14.8, 7.1, 1H)	1.74 (dd, 14.4, 2.4 , 1H)	1.68 (m, 1 H)
12	5.54 (t, 7.7, 1H)	4.91 (dd,10.0, 2.4,1H)	4.88 (dd, 9.7, 2.1, 1H)
15	-	1.63 (s, 3H)	1.60 (s, 3H)
15a	5.02 (s, 1H)	-	-
15b	4.93 (s, 1H)	-	-
17	3.51 (s, 2H)	3.67 (s, 2H)	3.65 (s, 2H)
OCH_3-17	3.38 (s, 3H)	3.42 (s, 1H, 3H)	3.40 (s, 3H)
18	1.78 (s, 3H)	1.63 (s, 3H)	1.63 (s, 3H)
19	1.88 (s, 3H)	1.63 (s, 3H)	1.63 (s, 3H)

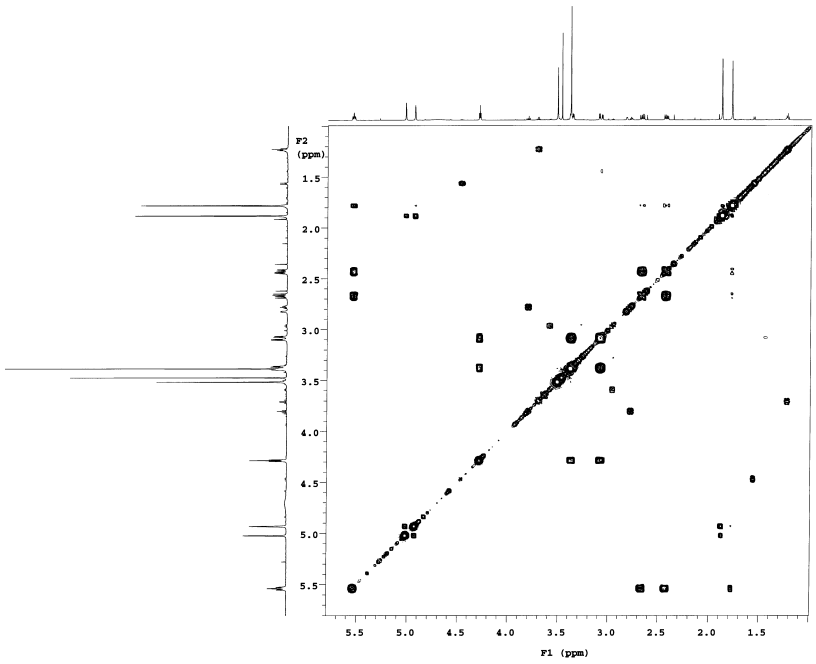
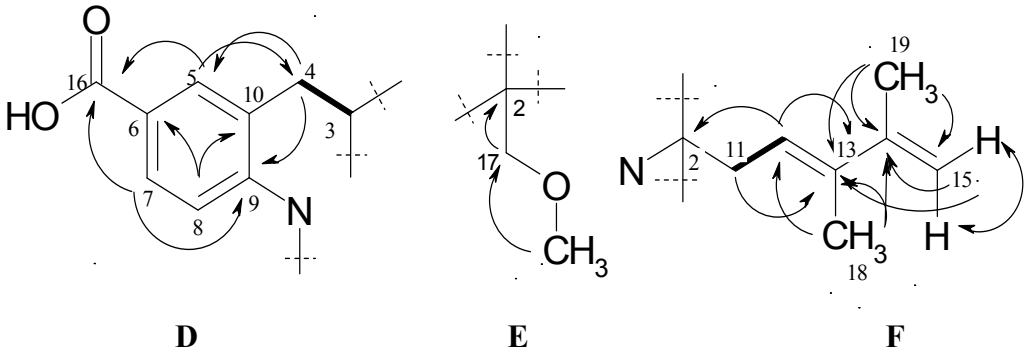


Figure 34. H,H COSY spectrum (600 MHz, $CDCl_3$) of ramthacin A (**44**).

Based on HMBC spectrum, two of the aromatic protons (H-5,7) showed a strong correlation with the carbonyl group (C-16) which located the carbonyl carbon at C-6 as well as the correlation from the methylene H-4 and the methine H-3 to the aromatic carbons C-5 and C-10, respectively, which connect fragment A and B together. The methyl groups H-18, H-19 as well as the geminal protons H-15 displayed correlations to C-13 and C-14, furthermore, the methyl H-18 showed correlations to C-11 and C-12, which resulted in fragment F. Through the correlations from the methylens CH_2-4, 11,17 and the methine H-12 to the quaternary carbon C-2 the following fragments could be connected to afford the structure of ramthacin A (**44**). The name ramthacin is derived from a Jordanian city situated in the north named "Ramtha" and "cin" came from the relevant compound virantmycin. As the bioactivity could not be measured due to less stability, it was named as -cin instead of -mycin.



D **E** **F**

Figure 35. Significant correlation observed in the H,H COSY (▬) and HMBC (→) spectra of ramthacin A (**44**).

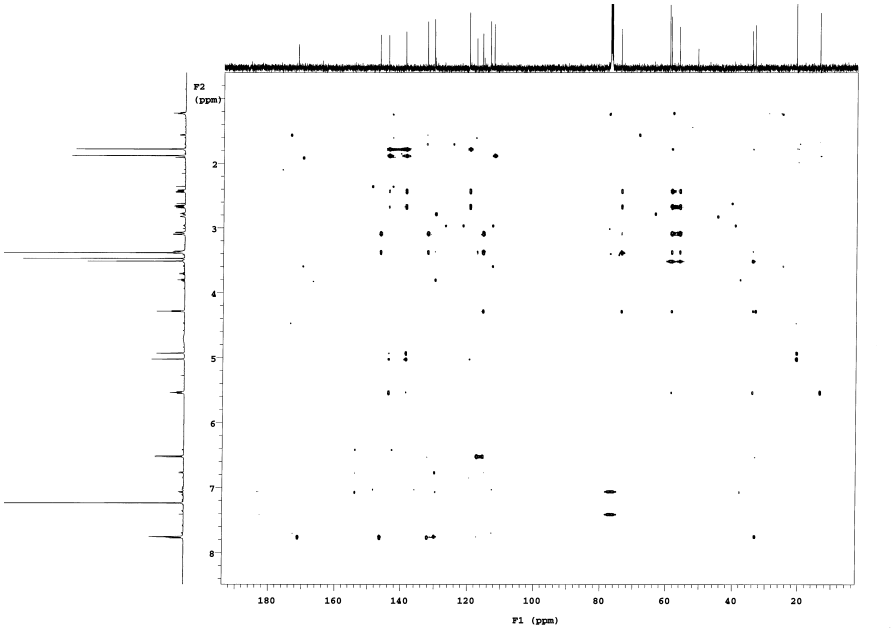
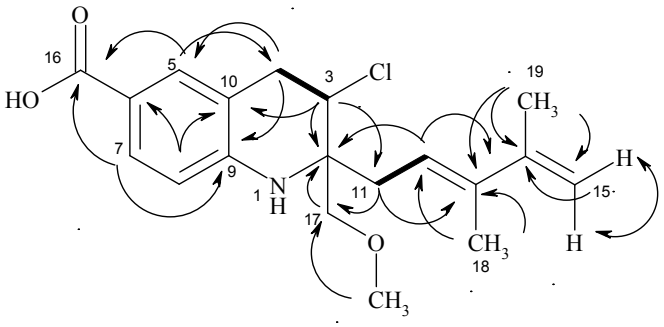


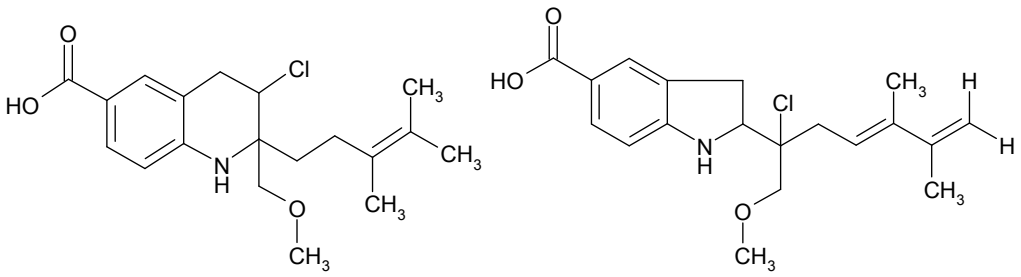
Figure 36. HMBC spectrum (600 MHz, $CDCl_3$) of ramthacin A (**44**).

To connect the mentioned fragments of ramthacin A (**44**) there were two possibilities, either as tetrahydroquinoline, like (-)-virantmycine (**45**) or as 2,3-dihydroindole **46**.[68] The structure of ramthacin A (**44**) was finally confirmed by comparison of the ^{13}C NMR data with those of (-)-virantmycin (**45**), which demonstrated a close similarity (see Table 5).



44

Figure 37. Selective H,H COSY (▬) and HMBC (→) correlations of ramthacin A (**44**).



45 **46**

5.1.3 Ramthacin B

The colourless oily ramthacin B (**47**) isolated from sub-fraction III (Figure 23) had a middle polarity. It showed the same behaviour like ramthacin A (**44**), was UV absorbing at 254 nm and gave a dark green colour with anisaldehyde/sulphuric acid, which changed to brown with the time after spraying the chromatogram. The molecular weight was established from ESI and EIMS to be *m/z* 367. HREI revealed the molecular formula of **47** as $C_{19}H_{26}NO_4Cl$, entailing seven double bond equivalents with one water molecule (Δm =18) more than in ramthacin A (**44**).

The ^1H NMR spectrum of compound **47** showed a high similarity to ramthacin A (**44**). The difference was observed in the olefinic region with the disappearance of the proton signals at δ 5.54, 5.02, 4.93 in ramthacin A and the appearance of an oxygenated methine at δ 4.91 (H-12) in the aliphatic region of ramthacin B (**47**). Addition-

ally, three methyls attached to sp^2 carbons were observed at 1.63 (H-15,17,18) instead of the two methyls in ramthacin A (**44**).

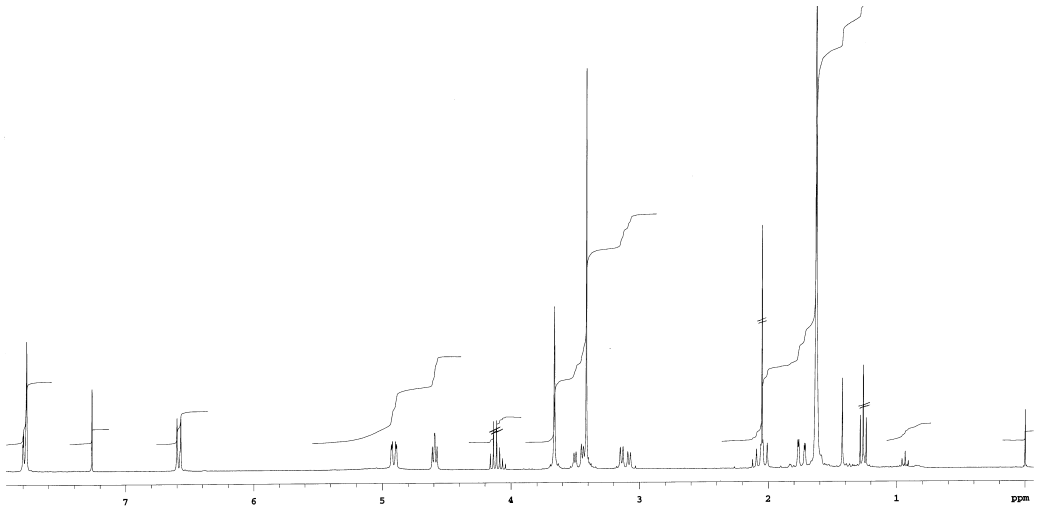


Figure 38. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of ramthacin B (**47**).

The ^{13}C NMR spectrum of **47** 8 sp^2 carbon signals were observed between δ 114.1-146.7, instead of 10 sp^2 carbons in ramthacin A. Additionally, three methyl carbon signals at δ 21.1, 19.5 and 12.1 were observed instead of the two present in ramthacin A (**44**).

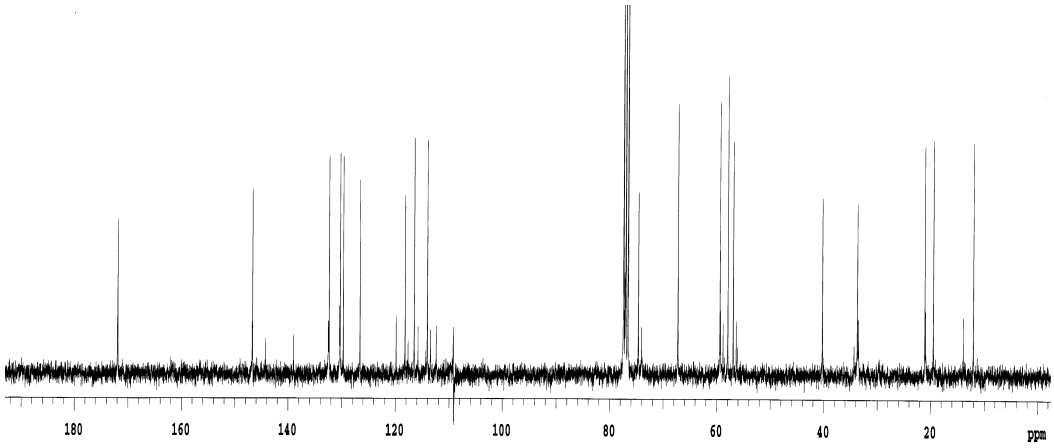
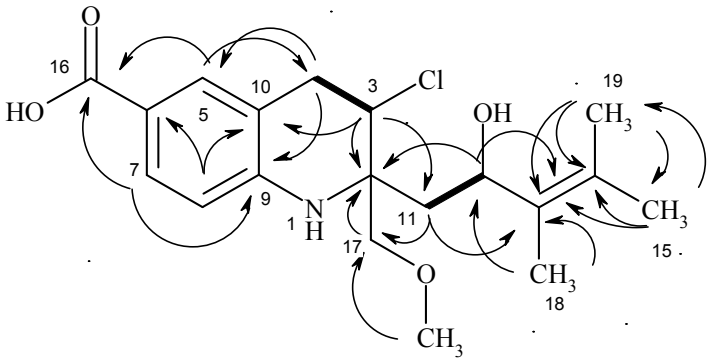


Figure 39. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of ramthacin B (**47**).



47

Figure 40. Selective H,H COSY (▬) and HMBC (→) connectivities of ramthacin B (**47**).

Ramthacin B (**47**), also a chlorine-containing compound similar to **44** isolated from the same fraction III. Its structure was confirmed by direct comparison with ramthacin A (**44**).

Table 5. Comparison of ^{13}C NMR chemical shifts of ramthacin A (**44**), B (**47**), and C (**48**) with (-)-virantmycin (**45**).

Position	(-)-virantmycin (**45**) δc	Ramthacin A (**44**) δc^a	Ramthacin B (**47**) δc^b	Ramthacin C (**48**) δc^a
2	58.0	58.8	58.0	57.7
3	56.0	56.4	57.0	76.3
4	33.6	33.5	33.8	27.5
5	132.4	132.5	132.5	132.7
6	117.7	117.6	118.2	117.8
7	130.4	130.4	130.3	130.1
8	113.5	113.5	114.1	114.1
9	147.2	146.8	146.7	147.5
10	116.0	115.8	116.5	117.2
11	33.5	34.2	40.3	41.2
12	27.8	119.9	67.3	66.8
13	128.8	139.1	129.7	130.1
14	126.5	144.2	126.6	125.9
15	19.9	112.4	19.5	19.5
16	171.9	171.5	171.8	171.8
17	74.1	74.1	74.7	74.3
18	18.8	13.9	21.1	21.1
19	20.6	21.0	12.1	12.1
17-OCH_3	59.4	59.3	59.4	59.4
3-OCH_3	-	-	-	56.9

[a] (125 MHz) [b] (75 MHz)

(-)-Virantmycin (**45**) is an unusual chlorinated tetrahydroquinoline that was isolated by Japanese workers[69-71] from a strain of *Streptomyces nitrosporeus*. It was found to possess both strong inhibitory activity against RNA, DNA viruses, and weak antifungal activity. Due to high biological interest these compounds were synthesised by different groups.[72-75] The microbial production of ramthacin A (**44**) was very less and the biological activity test for this compound could not be performed. Unfortunately, ramthacin B (**47**) and ramthacin C (**48**) decomposed in the NMR tube.

5.1.4 Ramthacin C

A third colourless oil with middle polarity named ramthacin C (**48**) was also isolated from the sub-fraction III (Figure 23) and showed the similar physical and chemical properties like ramthacins A (**44**) and B (**47**). The molecular weight was established from ESIMS to be *m/z* 363. The HRESIMS revealed the molecular formula of **48** as $C_{20}H_{29}NO_5$, entailing seven double bond equivalents and the absence of chlorine. The ^{1}H NMR showed a high similarity to ramthacin B (**47**) with an additional methoxy group was observed at δ3.42.

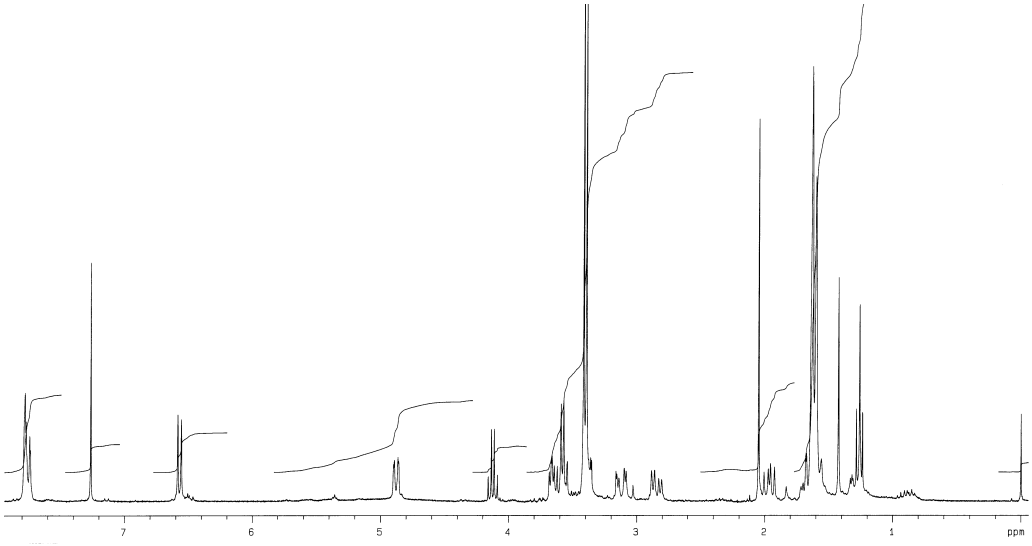


Figure 41. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) ramthacin C (**48**).

In the ^{13}C NMR spectrum the additional methoxy singlet was observed at δ 56.9. There was also a difference in the chemical shift of C-3 compared with ramthacin B (**47**), which was 76.3 instead of 57.0 indicating that the carbon is connected with a more electronegative atom like oxygen instead of chlorine.

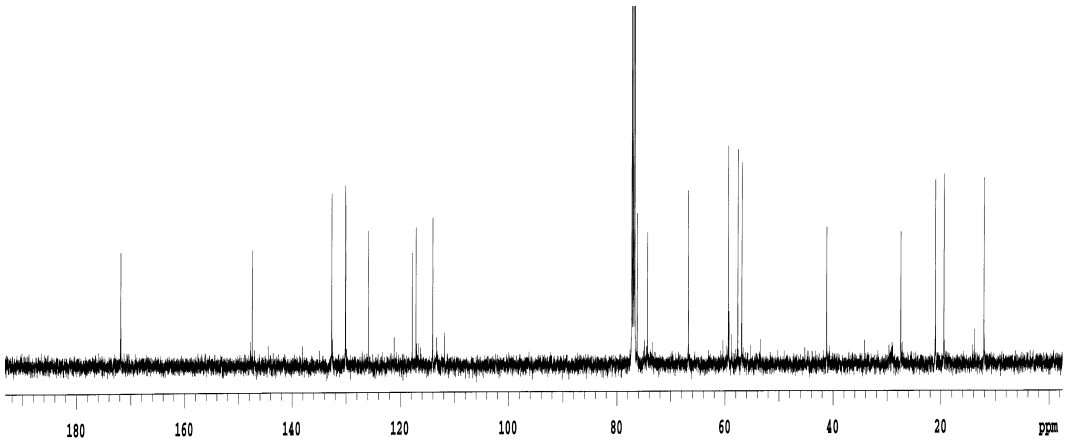
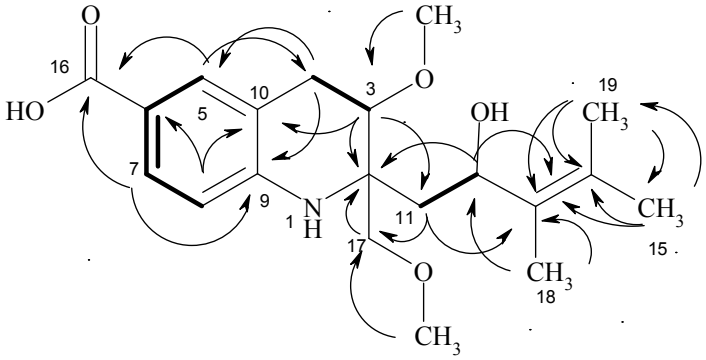


Figure 42. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of ramthacin C (**48**).



48

Figure 43. Selective H,H COSY (▬) HMBC (→) connectivities of ramthacin C (**48**).

The H,H COSY spectrum confirmed the presence of 1,2,4-trisubstituted benzene ring and $–CH_2CH-$ fragments. From the HMBC spectrum the correlations from H-4 and H-3 to the aromatic carbons C-9 and C-10 as well as the correlations from the methylenes H-4,11,12,17 to carbon C_q-4 were observed which were identical to ramthacin A (**44**) and B (**47**)

5.1.5 4-(5-Hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on

4-(5-Hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on (**49**) was isolated from the subfraction IIIb (Figure 23) using PTLC. It was obtained as colourless oil, was UV inactive and stained to violet after spraying with anisaldehyde/sulphuric acid. The ^1H NMR spectrum of **49** exhibited one singlet in the olefinic region at δ 5.87 attributed to an α-proton of an α,β-unsaturated carbonyl. In addition, two oxymethylene groups at δ 4.76 and 4.15, another two methylenes multiplet possibly connected to sp^2 at δ 2.52, 2.39 were observed. Finally, the spectrum showed two methyl proton signals connected to sp^2 carbons at δ 1.77.

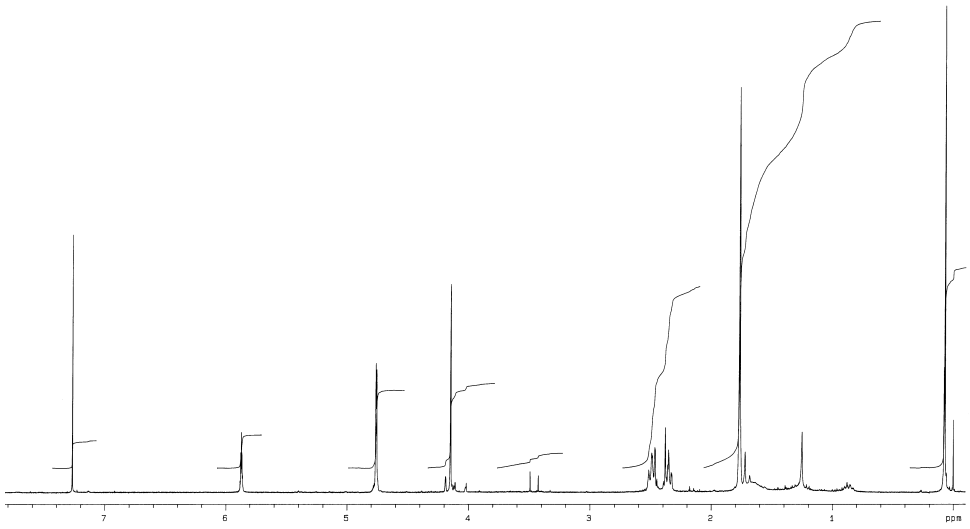


Figure 44. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4-(5-hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-one (**49**).

The ^{13}C NMR spectrum of **49** displayed 11 carbon signals, one carbonyl at δ 173.9, four olefinic at δ 169.9, due to a β-carbon of an α,β-unsaturated carbonyl, 115.6, 130.2, and at 129.9 respectively. In aliphatic region two methylene at 26.2, and 32.3, as well as, two methyl groups at δ 17.6, and 16.2 were observed. The ESI mass spectrum indicated the *pseudo*-molecular peak at *m/z* 219 $[M + Na]^+$, which fixed the molecular weight to 196 Dalton.

A search in AntiBase and the Chemical Abstracts using the above spectroscopic data gave no hit, which means that this compound is new. Thus compound **49** was subjected to 2D NMR experiments. The H,H COSY spectrum showed only a strong correlation between the two methylene groups at δ 2.52 and 2.39, as well as weak allyl correlations. The HMBC spectrum led to the correlation between the methylene protons at δ 5.87 and the carbonyl carbon at δ 173.9, (^3J), the quaternary carbon at δ 169.9 (^2J), olefinic carbon at δ 115.6 (^3J) and another methylene at δ 26.2 (^3J). The two methyls at δ 1.77 showed strong correlation with the two olefinic carbons at δ 130.2, 129.9. The 1D and 2D NMR data together with mass established the structure as 4-(5-hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on (**49**).

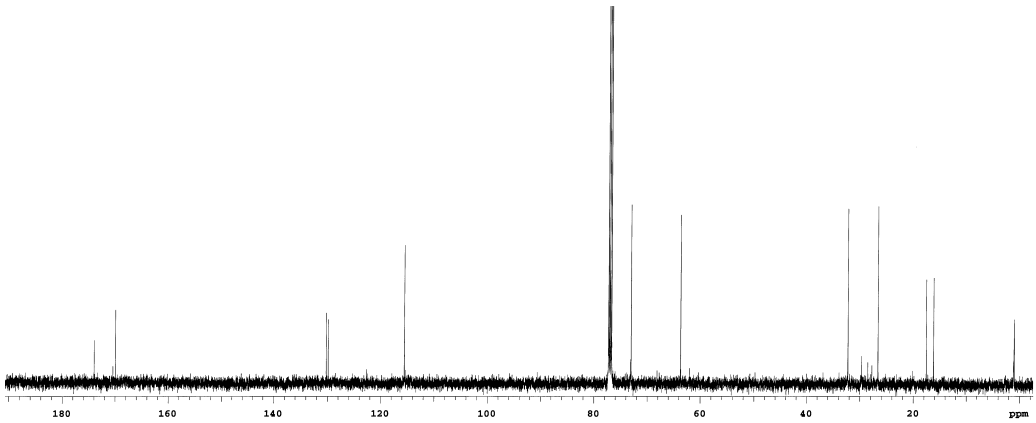
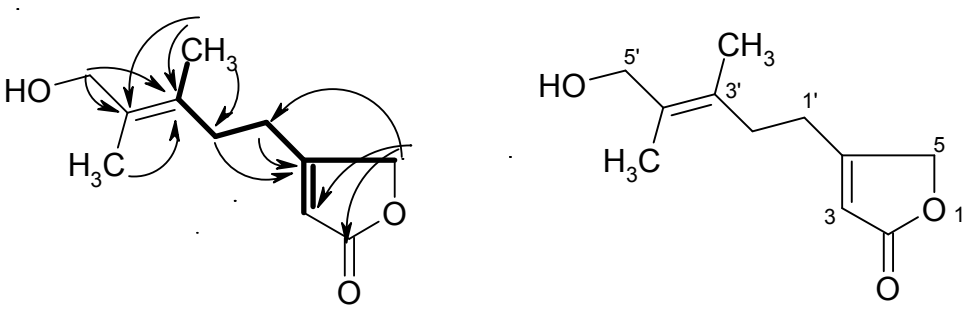


Figure 45. ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 4-(5-hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on (**49**).



49

Figure 46. H,H-COSY (▬) and HMBC (→) couplings of 4-(5-hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on (**49**).

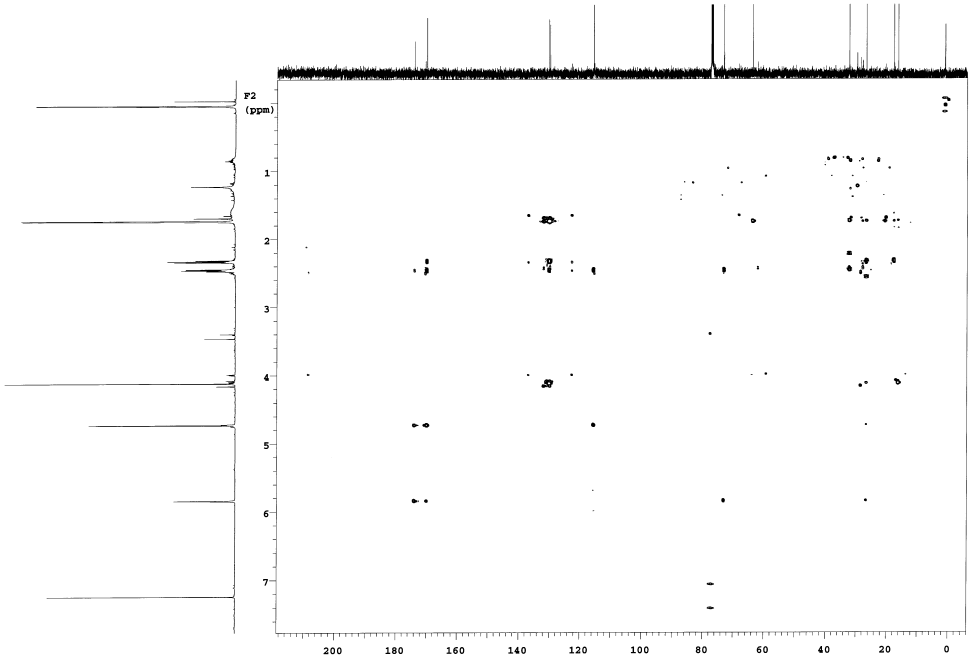
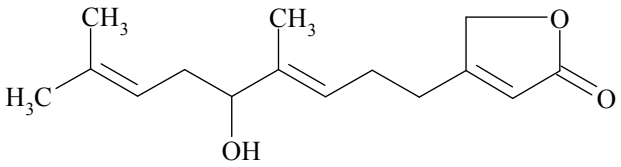
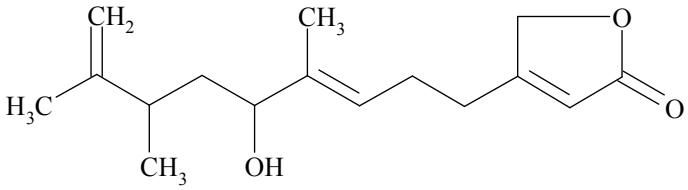


Figure 47. HMBC spectrum (600 MHz, $CDCl_3$) of 4-(5-hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on (**49**).

The structure of **49** was further confirmed by comparing the ^{1}H and ^{13}C NMR data with the similar published compounds; 8-hydroxy-3,7-dimethy-10-isopropyl-2Z,6E,10-dodecatrien-13,1-olide (**50**) and 8-hydroxy-3,7,10,11-tetramethyl-2Z,6E,11-dodecatrien-13,1-olide (**51**), which were isolated from *Gochnatia glutinosa*.[76]



50

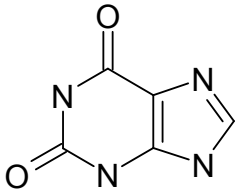


51

5.1.6 3,7-Dihyro-purin-2,6-dione (Xanthine)

Xanthine (**52**) was isolated by crystallization from fraction V (Figure 23) as a colourless solid (Figure 23), which showed UV absorbing bands at 254 nm in the highly polar region. The ^{1}H NMR spectrum of **52** showed one 1H singlet at δ 7.90. In addi-

tion, two broad signals belonging to three exchangeable protons were observed at δ 12.55 (2H) and 10.56 (1H). The EI mass spectrum showed the molecular ion peak at *m/z* 152 corresponding to the molecular formula $C_5H_4N_4O_2$. Using the above spectroscopic data as well as the molecular weight, a search in AntiBase led to xanthine (**52**). Xanthine (**52**) and its derivatives are forming a sub-group of alkaloids, are frequently used for their effect as mild stimulants and as bronchodilators, notably in treating the symptoms of asthma. Xanthine (**52**) was frequently isolated from microorganisms.



52

5.2 Marine-derived *Streptomyces* sp. B6924

The crude extract of the marine *Streptomyces* sp. B6924 exhibited antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* in the biological screening. The TLC screening revealed polar yellow to orange zones which did not changed to violet with diluted sodium hydroxide (2N) and gave dark orange colour with concentrated sulphuric acid indicating the presence of actinomycin, as well as several UV absorbing zones. The strain was cultivated on M_2^+ medium with 50% seawater in the scale of 25 L during seven days. The brown culture broth was mixed with Celite and filtered through a filter press, the water phase was loaded on a XAD-16 column and the adsorbing metabolites were eluted with methanol. The eluate was evaporated *in vacuo* and the aqueous residue extracted with ethyl acetate. The biomass was extracted with ethyl acetate and acetone. The dark brown oily crude extract was submitted to column chromatography on silica gel using a CH_2Cl_2/MeOH gradient.

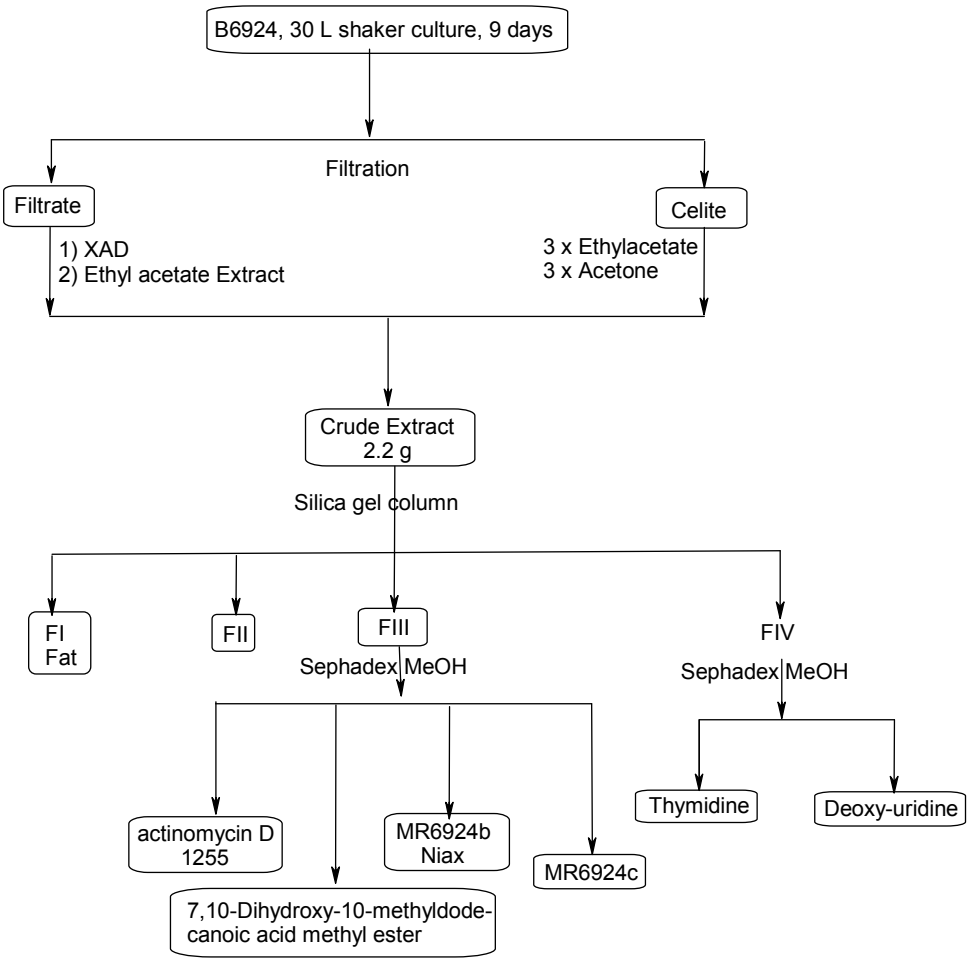


Figure 48. Work up scheme of marine *Streptomyces* sp. B6924.

5.2.1 7,10-Dihydroxy-10-methyldodecanoic acid methyl ester

Compound **53** was isolated as colourless oil from fraction III by Sephadex LH-20. It showed a 'white' colour reaction with anisaldehyde/sulphuric acid, i.e. the surrounding background changed the colour more than the spot itself. The ESI mass spectrum revealed *pseudo*molecular ion peaks at *m/z* 542 and 283 for $[2\ M + Na]^+$ and $[M + Na]^+$, respectively. HRESIMS of **53** afforded the formula $C_{14}H_{29}O_4$ with one double bond equivalent.

The ^1H NMR spectrum of **53** showed 26 aliphatic proton signals including a 3H singlet at δ 3.64 which pointed to a methoxy group, a 1H multiplet at δ 3.53 of an oxygenated methine, the 2H triplet at δ 2.33 of a methylene group connected with an sp^2 carbon and another methylene group at the other side, as well as six overlapped methylenes in the range of δ 1.51-1.35. Finally two methyl signals at δ 1.11 and 0.88 appeared as singlet and triplet, respectively. Based on the molecular formula it was clear that the molecule had two exchangeable protons due to two hydroxyl groups. A search in AntiBase and the Chemical Abstracts based on the above spectroscopic data

gave no results, pointing to a new natural product from microorganisms. To elucidate the structure of compound **53**, 2D NMR experiments were performed.

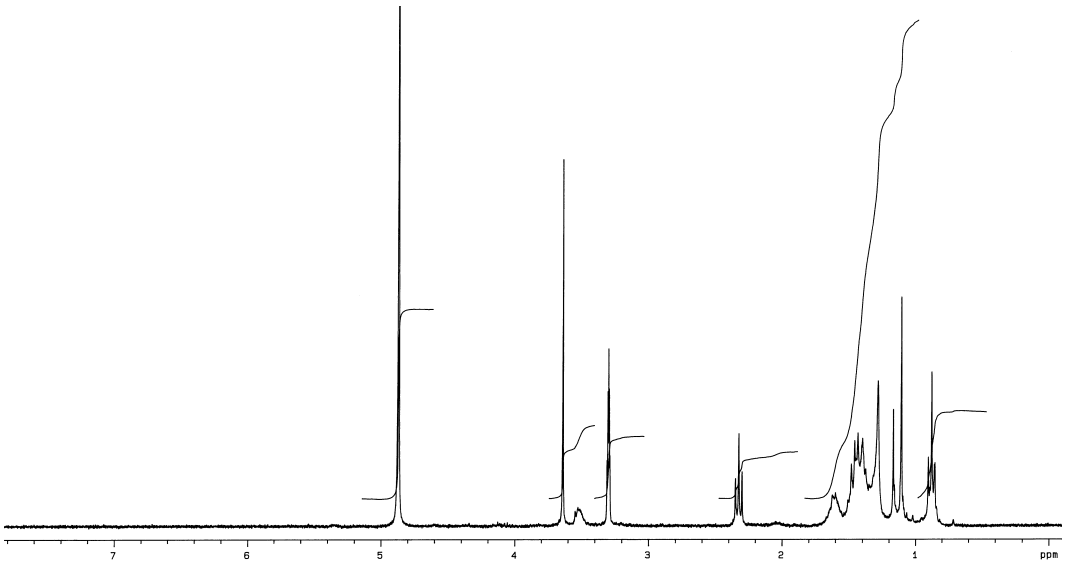


Figure 49. ^{1}H NMR spectrum (300 MHz, CD_3OD) 7,10-dihydroxy-10-methyldo–decanoic acid methyl ester (**53**).

The ^{13}C NMR spectrum of **53** contained 14 carbon resonances, which could be classified based on the analysis of the HSQC data as two quaternary carbon signals at δ 176.0 and 73.5 assigned to carbonyl (CO-1) and oxygenated carbon (C-10), respectively, one oxy-methine carbonyl signal at δ 72.2, a methoxy group at δ 52.0 as well as to eight methylene carbon signals between δ 42.2-21.1. Finally two methyl groups at δ 26.3 and 8.5 were observed.

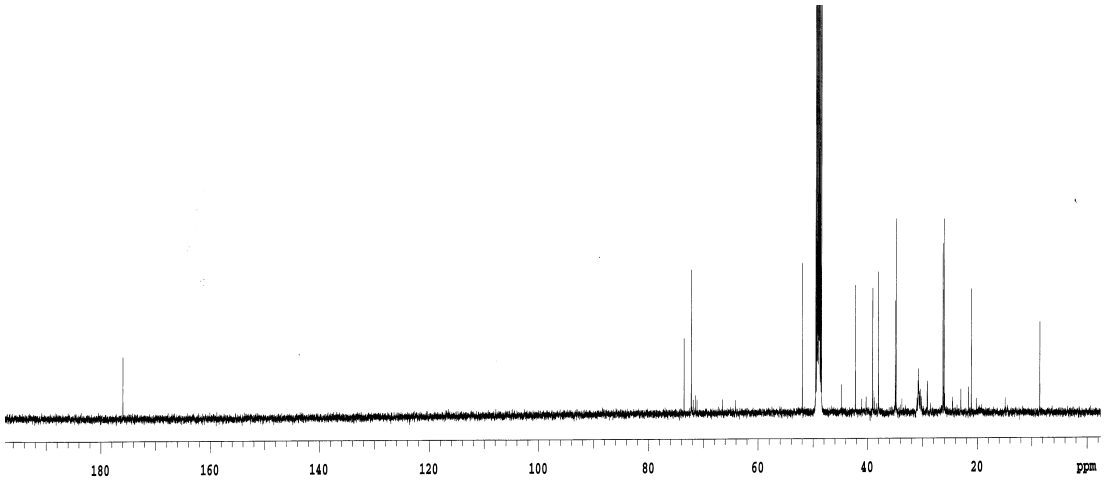


Figure 50. ^{13}C NMR spectrum (175 MHz, CD_3OD) of 7,10-dihydroxy-10-methyldodecanoic acid methyl ester (**53**).

The H,H COSY spectrum showed strong coupling between the methylene at δ 2.33 (H-2) and the methylene at δ 1.60 (H-3), as well as between the methyl proton at δ 0.88 and a methylene within the range of δ 1.51-1.35.

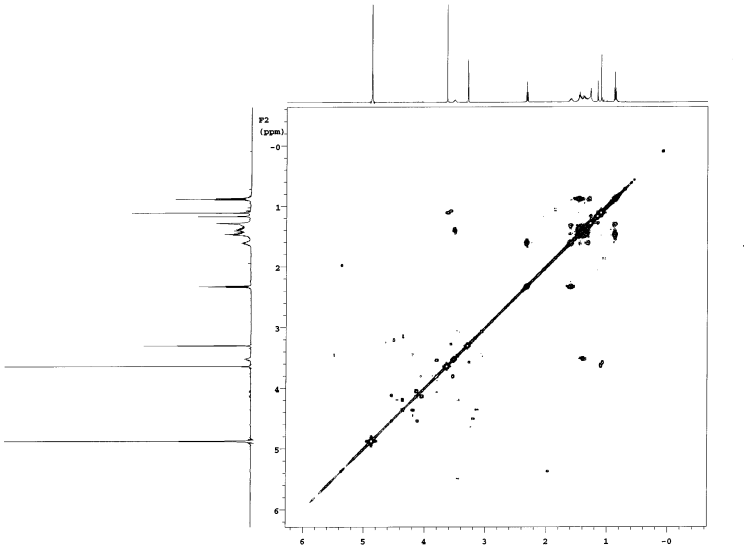
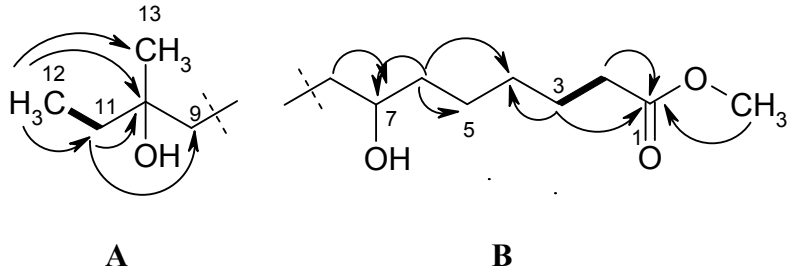


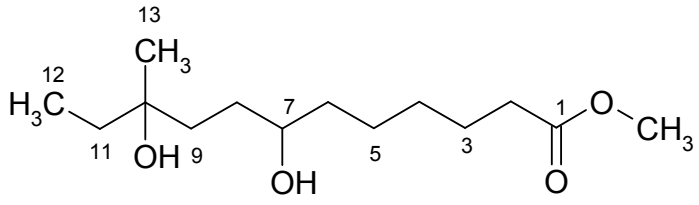
Figure 51. H,H COSY spectrum (600 MHz, CD_3OD) of 7,10-dihydroxy-10-methyldodecanoic acid methyl ester (**53**).

The HMBC gave further insight from the correlations between the methyl at δ 0.88 to carbons at δ 35.0 (C-11) and the oxygenated quaternary carbon at δ 73.5 (C-10). In addition the methylene at δ 1.48 (H-11) showed ^3J coupling with the methylene at δ 42.2 (C-9) and the methyl carbon at δ 26.3 (CH_3-10) which confirmed the partial structure A. The methyl of the ester at δ 3.64, the two methylenes at δ 2.33 (H-2) and 1.60 (H-3) exhibited strong correlation with the ester carbonyl at δ 176.0. The two methylene signals of CH_2-6 at δ 1.38 and 1.42 and of CH_2-8 at δ 1.40 showed ^2J coupling with hydroxylated carbon at δ 72.2 (C-7), while the former (CH_2-6) showed ^2J and ^3J coupling with methylenes at δ 26.1 (C-4) and 21.1 (CH_2-5), respectively, which established partial structure B. The methylene protons at δ 1.40 (H-8) displayed correlation with C-10, which established the connection of partial structure A and B through C-8 and C-9.



A **B**

Figure 52. Fragments resulted from HMBC (→) and H,H COSY (▬) spectra of **53**.



53

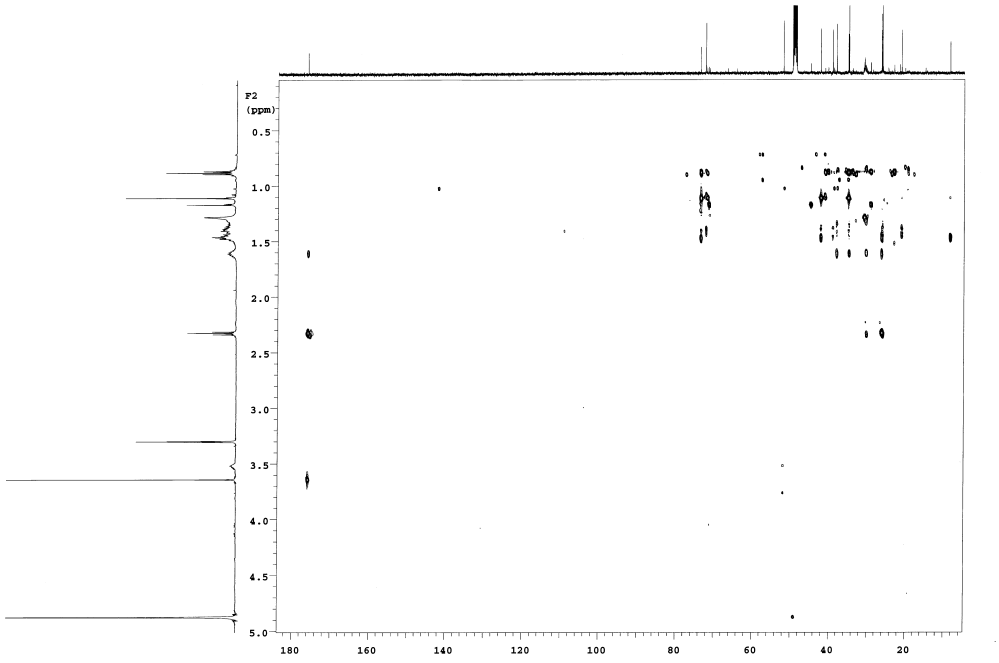


Figure 53. HMBC spectrum (600 MHz, CD_3OD) of 7,10-dihydroxy-10-methyldodecanoic acid methyl ester (**53**).

The 1D and 2D NMR established compound **53** as 7,10-dihydroxy-10-methyldodecanoic acid methyl ester which is a new secondary metabolite.

5.3 Marine *Streptomyces* sp. B8041

The crude extract of marine *Streptomyces* sp. B8041 showed weak activity against *Staphylococcus aureus* only. The chemical screening indicated a moderately polar zone, which showed no UV absorption, but turned to green with anisaldehyde/sulphuric acid. Several yellow and red compounds turned to red with sodium hydroxide, and to brown with sulphuric acid, pointing to *peri*-hydroxyquinones. Separation of the crude extract by silica gel and Sephadex LH-20 chromatography led to the isolation of two new compounds together with saptomycin A (**58**), β-indomycinone (**57**), and a mixture of two diketopiperazines.

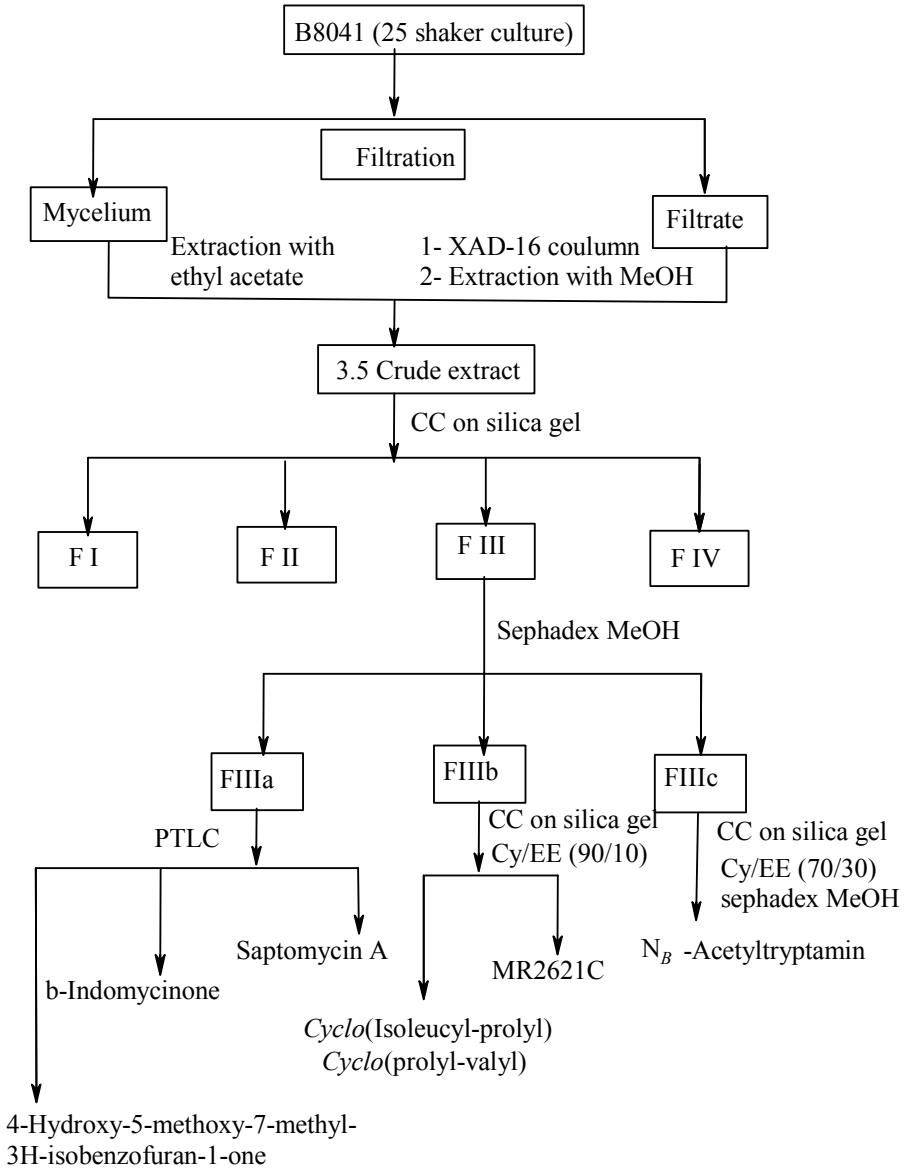


Figure 54. Work-up of the Marine *Streptomyces* sp. B8041.

5.3.1 4-Hydroxy-5-methoxy-7-methyl-3*H*-isobenzofuran-1-one

Yellow needles of **56** were obtained by chromatography of fraction III (Figure 54) on PTLC silica gel, which gave no colour reaction with anisaldehyde/sulphuric acid and heating. The molecular formula $C_{10}H_{10}O_4$ determined by HRESIMS indicated six double bonds equivalents.

The ^{1}H NMR spectrum of **56** showed one singlet in the aromatic region at δ 6.77 (H-6), the upfield shift of this signal indicated that the proton was in the *ortho* position to an electron-donating group. A broad singlet at δ 5.70 belongs to exchangeable H/D proton (4-OH) was observed. It also displayed signals attributed to one oxygen-

bearing methylene at δ 5.22 (H-3), and one oxygen-bearing methyl at δ 3.98 (5-OCH_3). Finally, a methyl at δ 2.61 (H-7) connected to sp^2 carbon was observed.

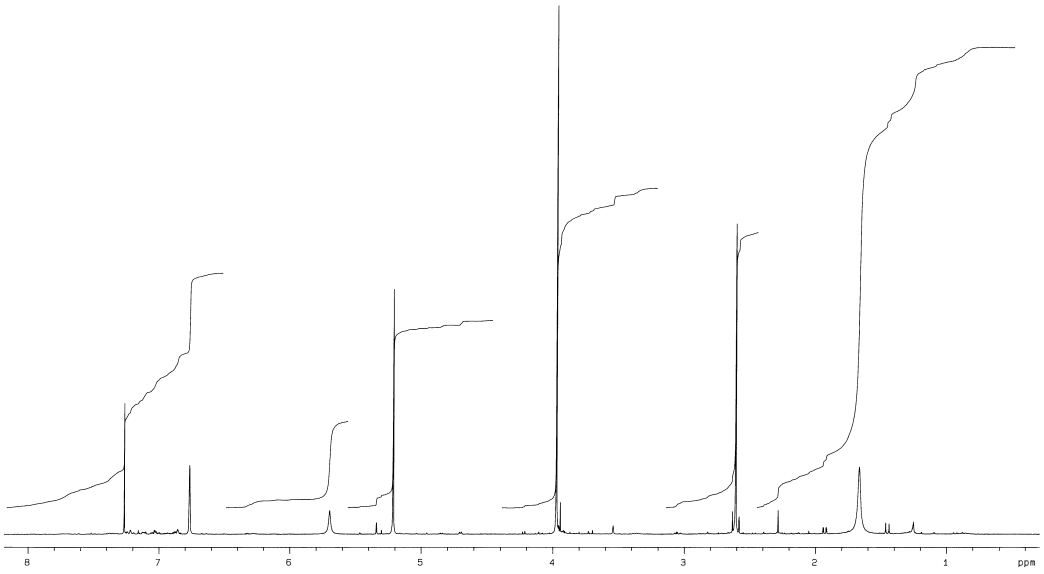


Figure 55. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 4-hydroxy-5-methoxy-7-methyl-3*H*-isobenzofuran-1-one (**56**).

The ^{13}C NMR spectrum of **56** showed signals of 10 carbons attributable to one carbonyl of acid ester or amide at δ 171.2, five quaternary carbons among them one oxygenated at δ 150.2 (C-5); one methine carbon at δ 113.4 (C-6) and one methylene carbon connected to oxygen at δ 66.6 (C-3). Additionally, two methyl carbon signals were observed: one methoxy at δ 56.5, and one connected to sp^2 carbon at δ 16.9. Searching in AntiBase using all the spectroscopic data led to two hits, 5-hydroxy-7-methoxy-6-methyl-1(3*H*)-isobenzofuranone (nidulol) (**54**) and silvaticol (**55**).

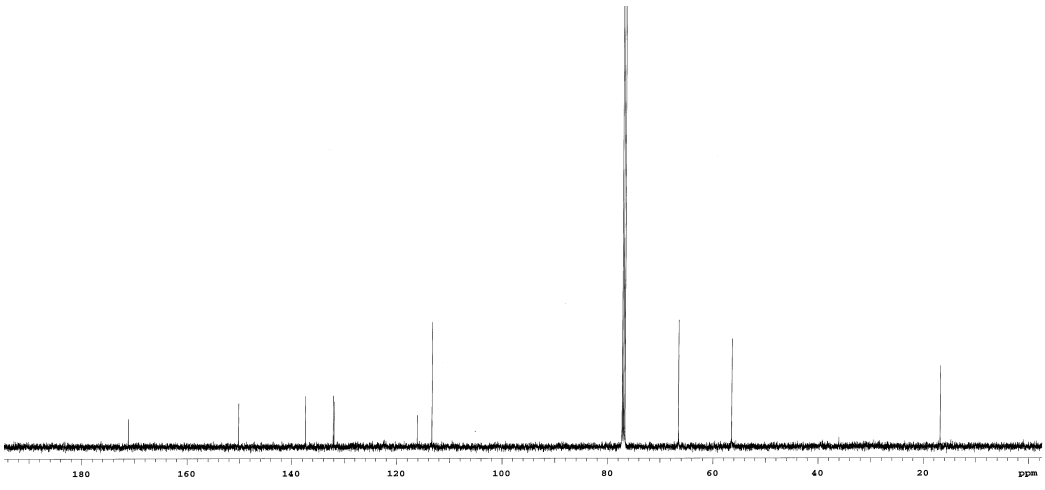
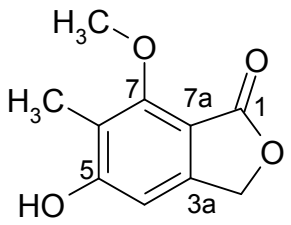
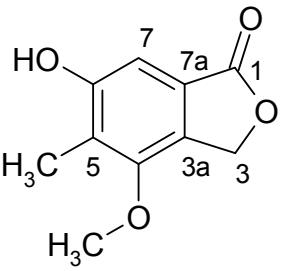


Figure 56. ^{13}C NMR spectrum, (CD_3OD, 125 MHz) of (**56**).



54

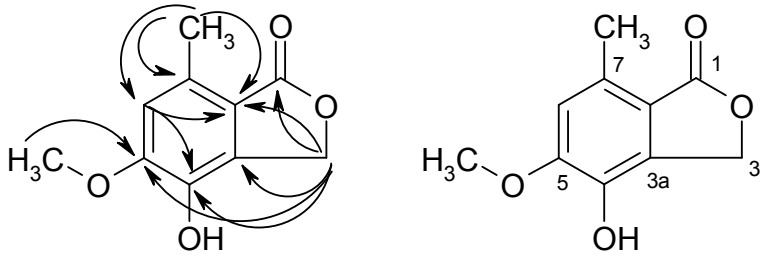


55

With the skeleton of a phthalide, 24 different structures are possible. To locate the position of the methyl, methoxy and hydroxyl groups, 2D NMR measurements were performed. From the HMBC experiment, the methylene singlet at δ 5.22 showed strong correlations with the quaternary carbon at δ 132.1 (C_q-3a), the carbon at δ 137.4 (C_q-4), and the carbon at δ 150.2 (C_q-5) which excluded structure **54** with its proton at C-4. The methyl group at C-7 displayed strong correlations with CH-6 (δ 113.4) and the C_q-7,7a (δ 132.0, 116.2) which also excluded structures **54** and **55** which would have displayed correlations between the methyl group and two oxygenated carbons.

According to these results, the structure of the isolated compound was neither that of structure **54** or **55**. For further confirmation the ^{1}H and ^{13}C NMR data of **56** were compared with those of **54** and **55** (Table 6): the difference between these compounds was evident. Further inspection of the HMBC spectra led to structure **56**, which is in good agreement with the downfield shift of the methyl signals (due to the carbonyl group) and the weakly chelated OH group. Irradiation into the methyl and the methoxy signal gave the expected nuclear Overhauser effects of the proton signal of H-6. As also an interaction between the methyl group and the hydroxy group were observed, the signals were additionally evaluated by means of COCON[77] : **56** was the only plausible result.

Compound **56** is new member of the phthalide group and is structurally related to silvaticol (**55**) and nidulol (**54**); the only difference is seen in the oxidation pattern of the phenyl ring. Nidulol (**54**) and silvaticol (**55**) have been isolated from *Aspergillus silvaticus* Fennell and Raper strain IFO 8173,[78,79] from *Emericella desertorum*, strain CBS 653.73[80] and from *Aspergillus duricaulis*.[81]



56

Figure 57. Selected HMBC correlations of 4-hydroxy-5-methoxy-7-methyl-3*H*-isobenzofuran-1-one (**56**).

Table 6. Comparison of ^{1}H and ^{13}C NMR data for 4-hydroxy-5-methoxy-7-methyl-3*H*-isobenzofuran-1-one (**56**), nidulol (**54**) silvaticol (**55**).

	4-hydroxy-5-methoxy-7-methyl-3*H*-isobenzofuran-1-one		Nidulol		Silvaticol	
Positio	$^{1}H^{a}$	$^{13}C^{a}$	$^{1}H^{b}$	$^{13}C^{c}$	$^{1}H^{b}$	$^{13}C^{c}$
1	-	171.2	-	173.1	-	171.1
3	5.22	66.6	5.15	69.1	5.39	69.0
C-3a	-	132.1		125.2		125.9
4	-	137.4	6.59	150.7		153.3
OCH_3-	-	-	-	-	3.91	59.3
OH-4	5.7	-	-	-	-	-
5	-	150.2		163.4		123.4
OCH_3-	3.98	56.5		-		158.3
CH_3-5	-				2.23	9.7
6	6.77	113.4		118.9		
CH_3-6	-		2.19	8.7		-
7	-	132.0		159.2	7.04	105.0
C-7a	-	116.2		109.3		127.2
CH_3-7	2.61	16.9				-
OCH_3-			4.08	62.1		

($^{a}CD_3ODD$, 300 MHz $^{b}CDCl_3$ ^{c}acetone-d_6)

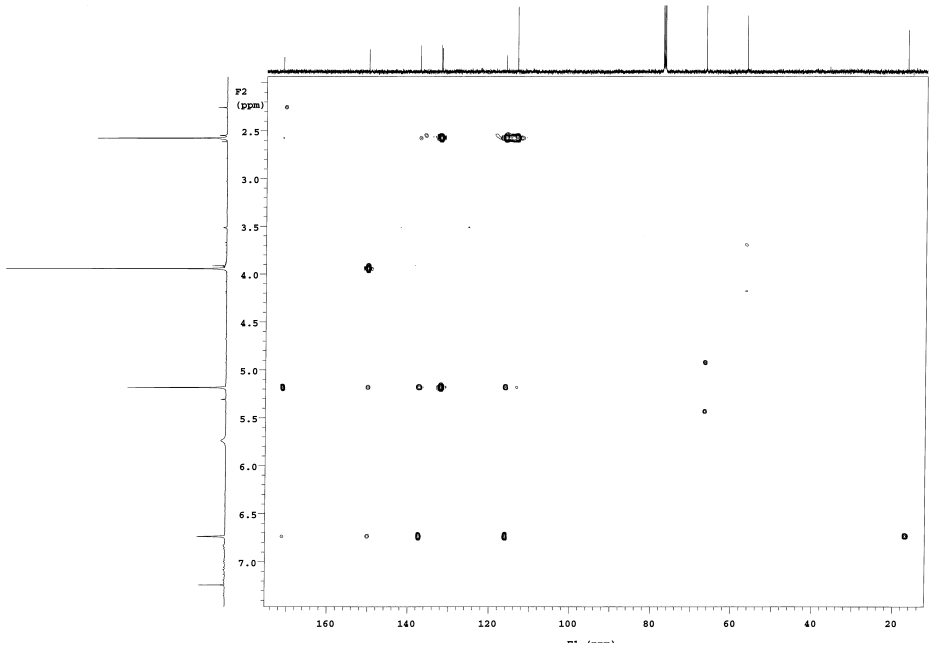
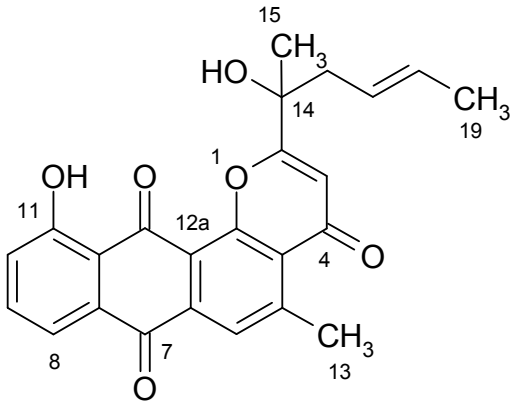


Figure 58. HMBC spectrum (CD_3OD, 300 MHz) of 4-hydroxy-5-methoxy-7-methyl-3*H*-isobenzofuran-1-one (**56**).

5.3.2 β-Indomycinone

Compound **57** was isolated from fraction III (Figure 54) as a yellow solid. It gave a red colour with sodium hydroxide, which is typical for *peri*-hydroxyquinones, and turned brown with sulphuric acid. The ^{1}H NMR spectrum of **57** showed a singlet at δ 12.85 indicating a chelated hydroxy group. Three protons were present at δ 7.83 (H-8), 7.68 (H-9) and 7.38 (H-10), as doublet, triplet, and doublet, respectively, corresponding to a 1,2,3-trisubstituted aromatic ring. In the olefinic region, two multiplets appeared at δ 5.74 (H-18) and 5.39 (H-17), due to the allylic protons of a methyl and methylene groups. In the aliphatic region, the singlet of a methyl group at δ 3.02 (H-13) in *peri*-position to the carbonyl (C-4) and two methylene protons of an ABX system were detected at δ 2.91 and 2.78. Finally, two methyl groups were present at δ 1.68 and 1.65 respectively: one as singlet, and the other as doublet of doublet due to the allylic coupling with H-17. The ESI mass spectrum of **57** displayed signals at *m/z* 403 $[M - H]^-$, 829 $[2 M - 2 H + Na]^-$. According to these spectroscopic data and a search in AntiBase this compound was determined as β-indomycinone (**57**) and identification was further confirmed by comparison with data of an authentic sample.[82,83]



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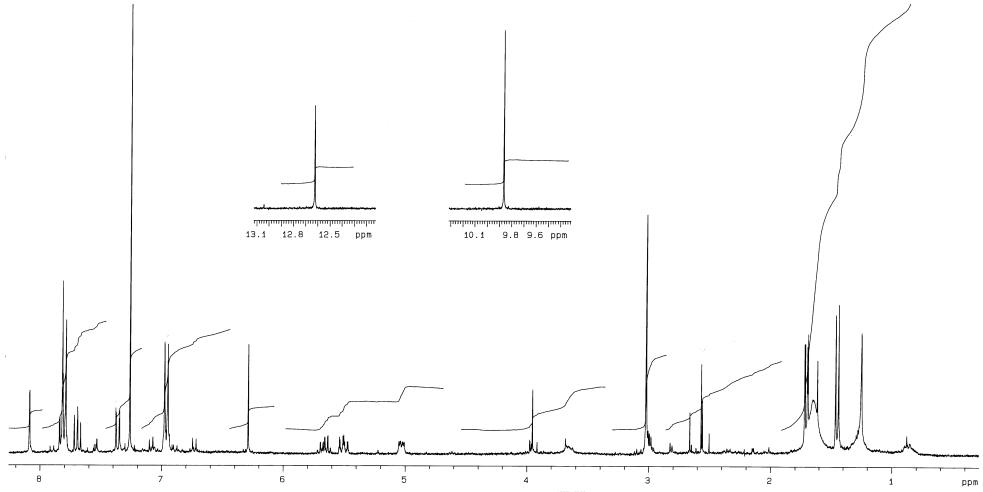


Figure 59. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of β-indomycinone (**57**).

Compound **57** was isolated by Brockman in 1980[84] and later by Schumacher and Nadig.[85,82] *β*-Indomycinone exhibited antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus*, *C. albicans* and *Streptomyces* sp. Tü 824 and showed antioxidant activity.[86]

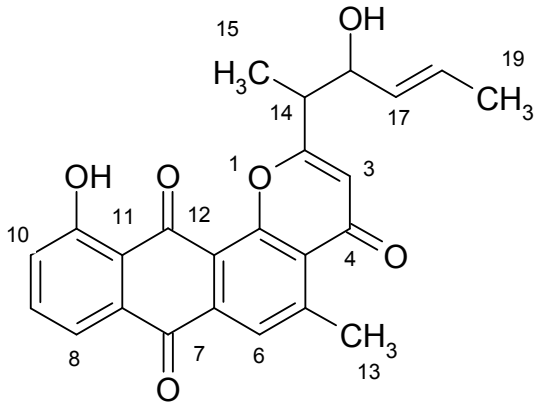
5.3.3 Saptomycin A

Compound **58** was also identified as *peri*-hydroxyquinone according to the test with sodium hydroxide. It showed close similarity with β-indomycinone (**57**), and was also isolated as a yellow solid from the same fraction, revealing similar physical and chemical properties as β-indomycinone (**57**).

In the ^{1}H NMR spectrum, a singlet of a *peri*-hydroxy group was present at *δ* 12.64. The aromatic region showed a 1H singlet at *δ* 8.09, three aromatic protons at *δ* 7.83, 7.69 and 7.36 of an 1,2,3-trisubstituted aromatic ring, a singlet at *δ* 6.28 (H-3), in addition to the singlet of a *peri*-methyl group at *δ* 3.02 (CH_3-13). Two multiplets of an olefinic double bond were detected at *δ* 5.65 and 5.50. In addition, two methine pro-

tons were displayed at δ 5.01 and 2.98, the former possibly oxygenated, while the latter connected to sp^2 carbon. Finally, at highest field, two methyl doublets were detected at δ 1.71 and 1.45.

The molecular weight of compound **58** was deduced as 404 Dalton by an ESI mass spectrum. The corresponding molecular formula of compound **58** was determined as $C_{24}H_{20}O_6$, based on the Rule of 13.[87] Finally, from the spectroscopic data and molecular formula of compound **58**, as well as a search in AntiBase, saptomycin A (**58**) was identified. This compound was further confirmed by comparing the data with those in the literature.[88]



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Saptomycin A is one of the members of the pluramycin family was isolated from *Streptomyces* sp. HP530 and it inhibited the growth of the *Bacillus subtilis* M45 a recombination deficient strain.[88,89] Pluramycin belongs to a family of polyketides containing the 4*H*-anthra[1,2-b]pyran-4,7,12-trione moiety and C-glycoside substituents, which are *Streptomyces*-derived natural product. The pluramycins were found to have antimicrobial and anticancer activity.[90]

5.3.4 *Cyclo*(isoleucyl-prolyl), *Cis-cyclo*(prolyl-valyl)

During the purification of subfraction IIIb (Figure 54) a mixture of two diketopiperazines was isolated as colourless solid. They showed a violet colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR displayed four methine multiplets at δ 4.19 (2H), 4.07 (1H), 4.03 (1H), which could be oxygenated, between two nitrogen atoms, or one nitrogen and one sp^2 carbon. Furthermore, two methylene multiplets between δ 3.60-3.53 (H-9), two methine multiplets at δ 2.48 and 2.13 (H-10) and a set of overlapped multiplets due to six methylenes between δ 2.40-1.20 were observed. In addition, two methyl groups were present at δ 1.06 and 0.92 as a doublet and triplet, respectively, as well as two equivalent methyl doublets at δ 1.09 and 0.92 of an isopropyl system. Compound **59** was shown to have the molecular formula $C_{11}H_{18}N_2O_2$

by HRESIMS (*m/z* 209.12959, $[M - H]^-$), while the molecular ion peak for **60** was not observed in the ESI mass spectra.

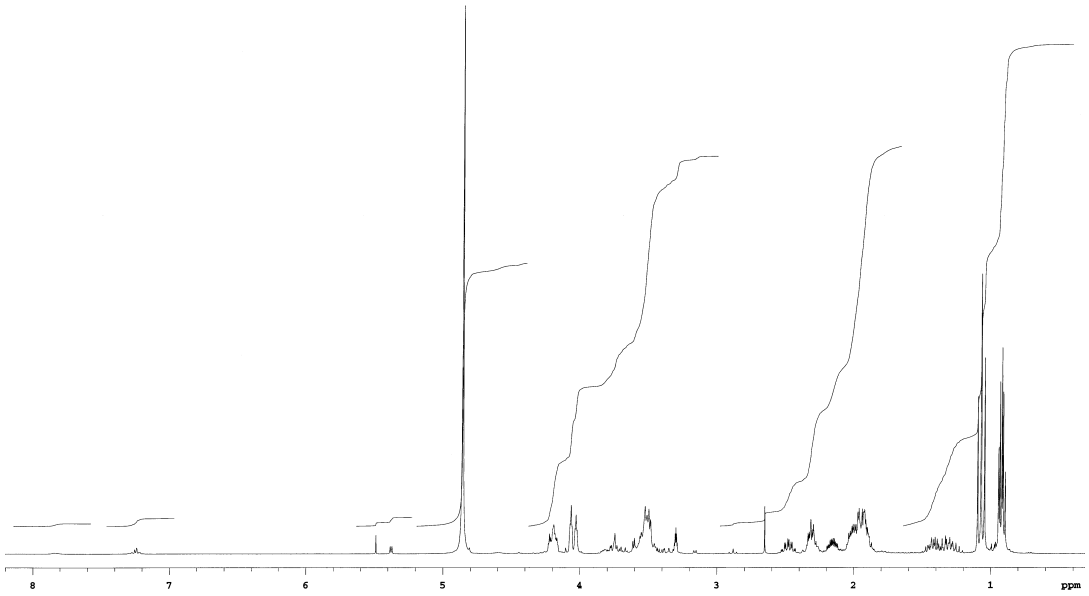
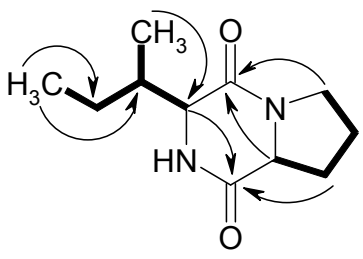


Figure 60. ^{1}H NMR spectrum (CD_3OD, 300 MHz) of *cyclo*(isoleucyl-prolyl) (**59**) and *cis-cyclo*(prolyl-valyl) (**60**).

The ^{13}C NMR spectrum displayed four carbonyl groups at δ 171.3, 171.1 and 166.1 (2 CO), four methine groups linked to a hetero atom at δ 60.2, 60.0, 58.7 (2CH) and two methylene carbons at δ 44.8. Furthermore, another two methines at δ 35.7 and 28.5 as well as another five methylene signals at 21.9 (2 CH_2), 24.1 and 28.2 (2 CH_2) were observed. Finally, four methyl signals at δ 17.5 and 15.3 belonging to the isopropyl group previously mentioned, as well as to 14.2 and 11.3 were observed. This mixture was subjected to 2D NMR experiments and careful interpretation of the data led to *cyclo*(prolyl-isoleucyl) (**59**) and *cyclo*(prolyl-valyl) (**60**). Compound **60** was confirmed as *cis-cyclo*(prolyl-valyl) by comparison with the authentic spectra.



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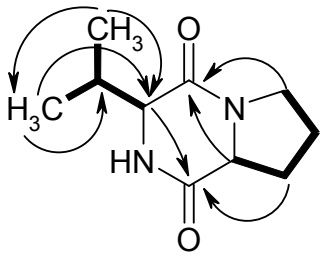

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Figure 61. Selected H,H COSY (—) and HMBC (→) correlations of *cyclo*(isoleucyl-prolyl) (**59**) and *cis-cyclo*(prolyl-valyl) (**60**).

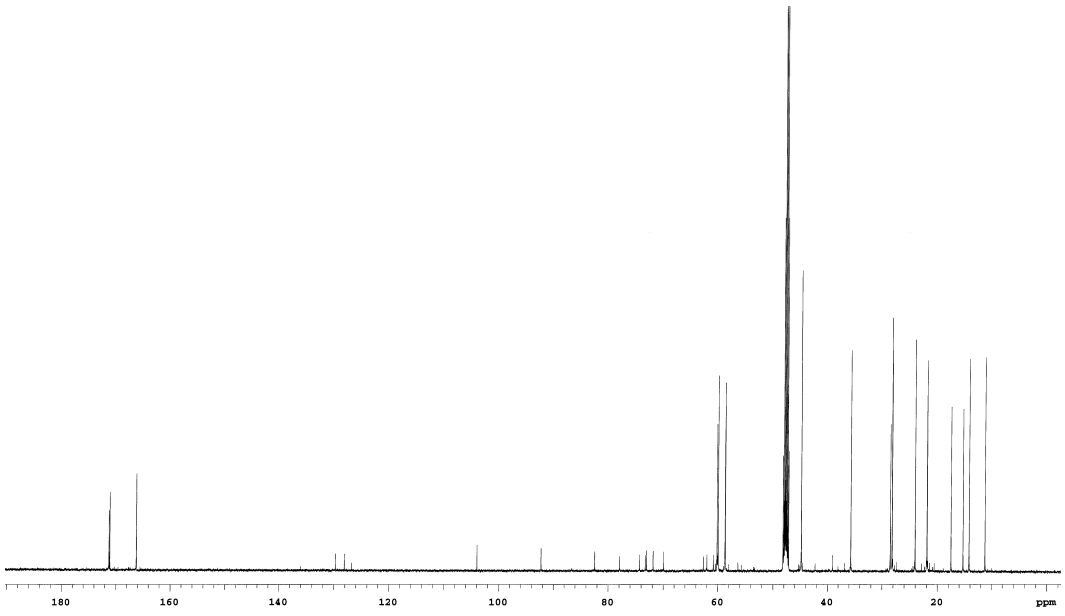
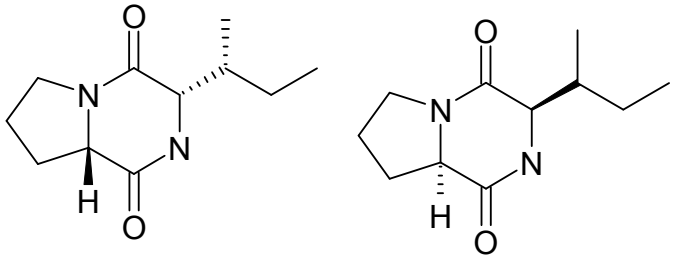
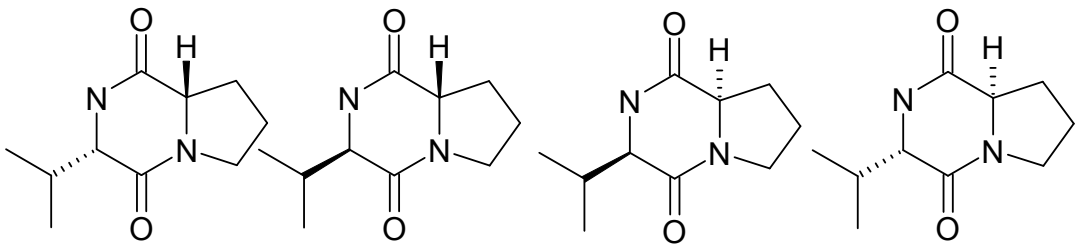


Figure 62. ^{13}C NMR spectrum (CD_3OD, 300 MHz) of *cyclo*(isoleucyl-prolyl) (**59**) and *cis*-*cyclo*(prolyl-valyl) (**60**).

Four different stereoisomers are possible for both compounds. In the case of **60**, all four are known as natural products, while the *trans* isomers of *cyclo*(isoleucyl-prolyl) have not been described so far from nature, and only *cyclo*(L-prolyl-L-isoleucyl) (**59a**) and *cyclo*(D-isoleucyl-D-prolyl) (**59b**) were mentioned in the literature. To confirm the stereochemistry of the isolated compounds, usually the optical rotation must be measured. But, as these compounds were isolated as a mixture and are commonly isolated compounds, no further measurements were performed.



59 **59**



60a **60b** **60c** **60d**

Cyclo(L-isoleucyl-L-prolyl) (**59a**) has been isolated from the culture media of *Vibrio parahaemolyticus*, which was isolated from the toxic mucus of the box fish[91] and from sponge-associated bacterium *Pseudomonas aeruginosa* Bio232; it had no antibiotic or cytotoxic activity.[92] *Cyclo*(D-isoleucyl-D-prolyl) (**59b**) and *cyclo*(D-prolyl-D-valyl) (**7c**) were isolated from bacterial strains CF-20 (CECT5719) and C-148, which have been isolated from larvae of mollusks; they showed strong antibiotic activity against *Vibrio anguillarum*.[93] *Cyclo*(L-prolyl-L-valyl) (**60d**) isolated from *Streptomyces* sp. strain ML 1532 had cytotoxicity against SV40-transformed cells.[94] Finally *cyclo*(D-prolyl-L-valyl) has been isolated from *Aspergillus* sp. F70609, and could inhibit the activity of β-glucosidase.[95] Recently some L,L-diketopiperazines have been identified as bacterial *quorum-sensing* compounds.[96,97] Gram negative bacteria normally use low-molecular weight compounds for the cell-to-cell communication which allows the bacteria to regulate gene expression in response to population density.

5.3.5 MR2621C

Compound **61** was isolated as colourless oil from sub fraction IIIb (Figure 54) by silica gel column chromatography. It showed no UV active zone and gave a green colour reaction with anisaldehyde/sulphuric acid on heating. ^{1}H NMR spectrum showed no proton signals in both aromatic and olefinic regions, however, there were two oxygenated methylene protons near chiral centre at δ 4.52 (Ha-3) and 4.12 (Hb-3), 4.15 (Ha-4) and 3.82 (Hb-4), respectively. Three methine protons: one oxygenated at δ 4.03, one at 3.34 and the latter sp^2 bound at δ 3.16, as well as three methylene protons in the range of δ 2.52-1.51 and one methyl triplet at δ 0.91 were observed.

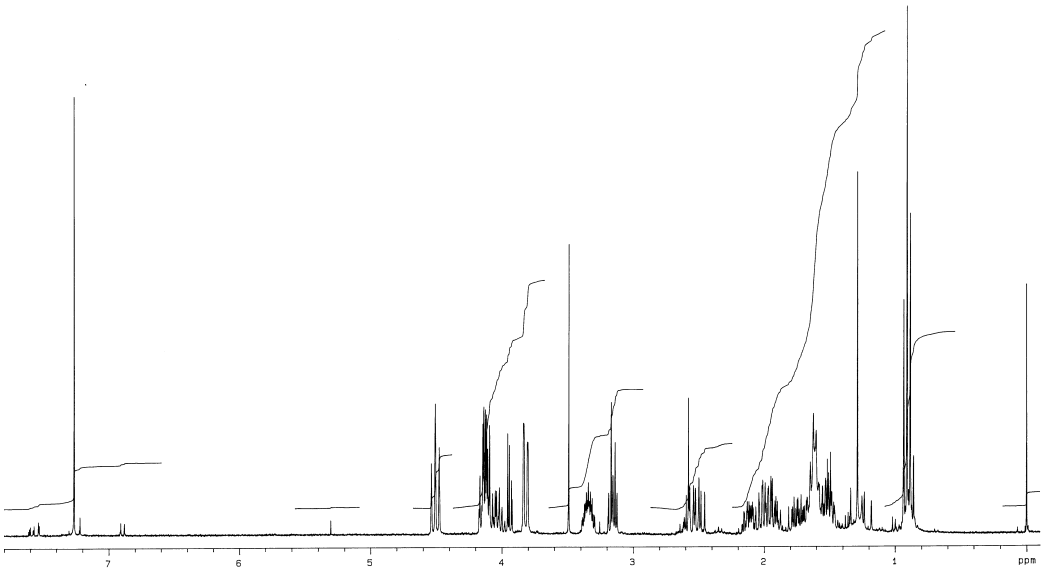


Figure 63. ^{1}H NMR spectrum, ($CDCl_3$, 300 MHz) of MR2621C (**61**).

In the ^{13}C NMR spectrum, there were 11 carbon signals visible; one carbonyl at δ 175.5, one quaternary carbon at δ 114.5 assigned to an acetal carbon, three oxygenated carbons at δ 82.3, 73.2 and 71.2, two methine carbon signals at δ 51.6 and 39.3 as well as three methylene carbon signals at δ 32.5, 30.0 and 29.8 and one methyl carbon signal at δ 10.1. The CI mass spectrum revealed the molecular ion peak at *m/z* 230 $[M + NH_4]^+$ and HRESIMS showed the molecular formula of $C_{11}H_{16}O_4$. Searching in AntiBase and the Chemical Abstracts using the above spectroscopic data and the molecular formula gave no hits indicating that this compound was new.

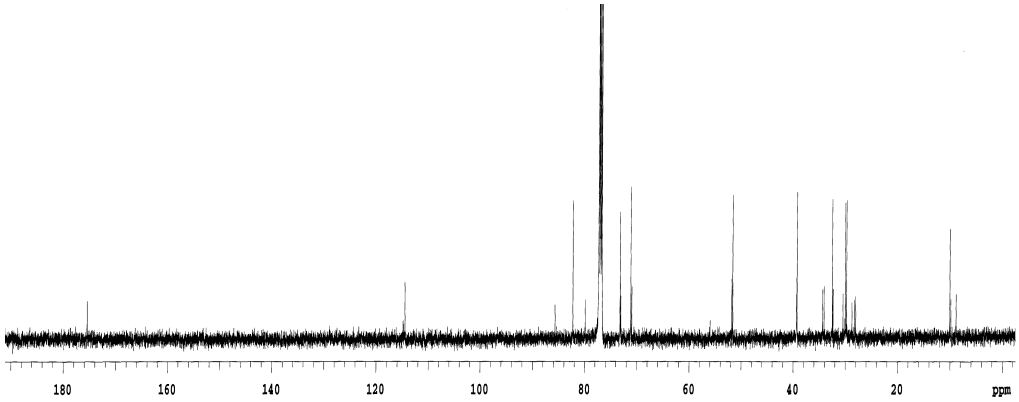
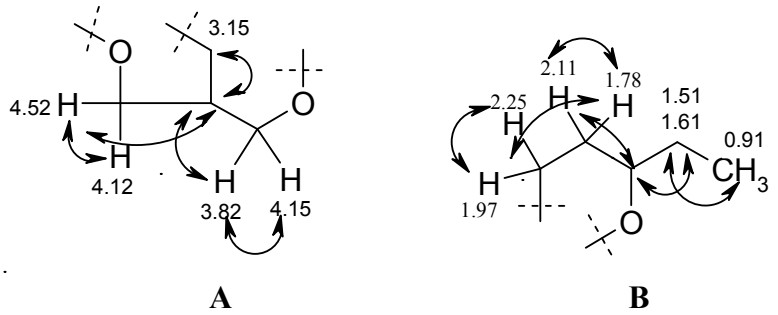


Figure 64. ^1H NMR spectrum, ($CDCl_3$, 300 MHz) of MR2621C (**61**).

To elucidate the structure of this compound 2D NMR experiments were performed. The H,H COSY spectrum showed strong correlation between the methine proton at δ 3.34 (H-3a) and the two methylene protons at δ 4.52 (Ha-3) and 4.12 (Hb-3), 4.15 (Ha-4) and 3.82 (Hb-4), and with the methine proton at δ 3.17 (H-5b). Furthermore, the oxygenated methine at δ 4.03 (H-7a) correlated with the methylene protons at δ 2.11 (Ha-7), 1.78 (Hb-7) and with the methylene at δ 1.61 (Ha-8), 151 (Hb8) which in turn correlated with methyl group at δ 0.91. This led to two fragments A and B.



A **B**

Figure 65. H,H COSY (—) fragments of MR2621C (**61**).

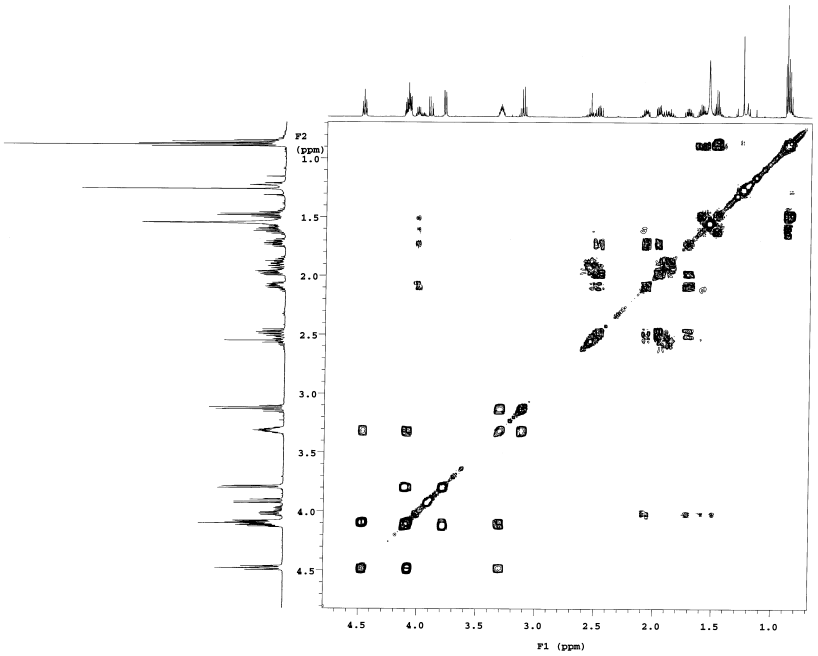


Figure 66. H, H COSY NMR spectrum, ($CDCl_3$, 600 MHz) of MR2621C (**61**).

The HMBC spectra displayed strong correlations of the methylene protons (δ 4.52, 4.12) and the methine proton (δ 3.1) with the carbonyl C-1 (δ 175.5), which resulted in a γ-lactone. The multiplet of H_b-4 (δ 3.82) exhibited ^3J correlation with C-3 (73.2), C-5b (51.6) and C_q-5a (114.5). Furthermore, protons H-5b (3.15), H-6 (2.25,1.97), and H-7 (2.11) exhibited correlations to the quaternary carbon (114.5), which confirmed the presence of a five-membered spiro ring. Additionally, the protons H_b-6 (1.97), H_b-7 (1.78), H-8 (1.61, 1.51) and the methyl protons H-9 (0.91) showed strong correlation to methine carbon C-7a (82.3), which resulted at the end in MR2621C (**61**).

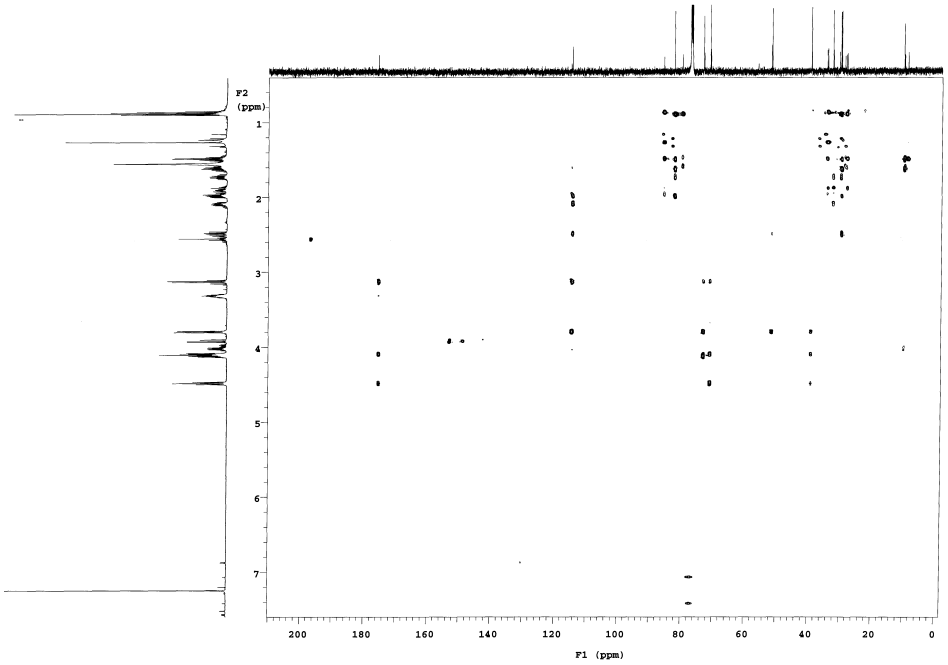
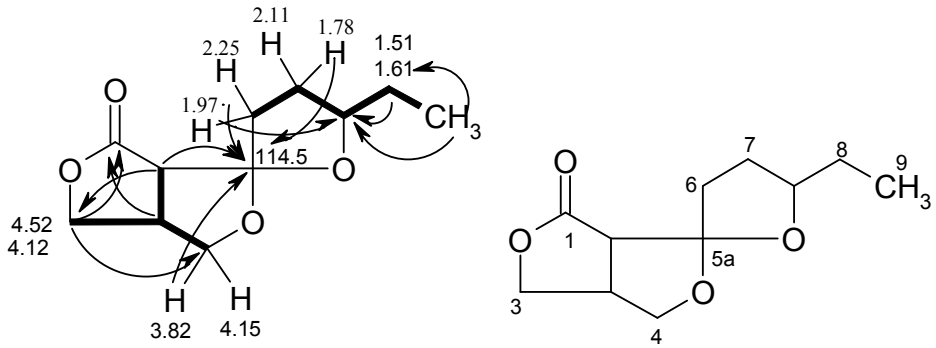


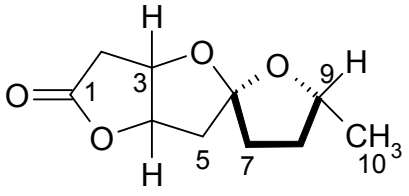
Figure 67. HMBC NMR spectrum, ($CDCl_3$, 600 MHz) of MR2621C (**61**).



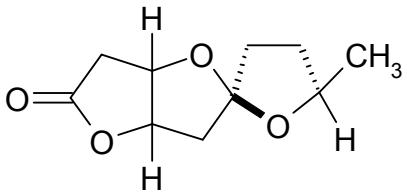
61

Figure 68. H,H COSY (▬) and selected HMBC (→) correlations of MR2621C (**61**).

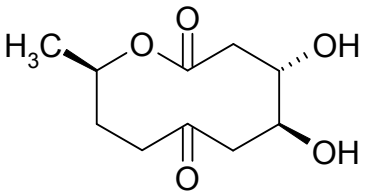
Searching in the Chemical Abstracts for similar compounds led to two related structures, cephalosporolides E (**62**) and F (**63**), which have been isolated from the fungi *Cephalosporium aphidicola* in 1985,[98] and *Cordyceps militaris* BCC 2816.[99] Hanson and co-workers reported that cephalosporolide E (**62**) and F (**63**) were possibly formed by hydrolysis, relactonization and then acetal formation of cephalosporolide C (**64**).[98]



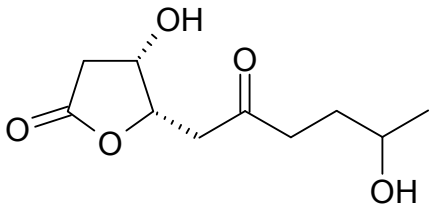
62



63



64



65

In 2005 Oller-López and co-workers[100] proved that bassianolone (**65**) was the true precursor of both cephalosporolides E (**62**) and F (**63**) when they passed it through a pad of silica gel. According to these finding 3-(4-hydroxy-hexanoyl)-4-hydroxymethyl-dihydro-furan-2-one (**66**) could be the precursor of **61**. The spectroscopic data of **61** were compared with those of cephalosporolide E (**62**) and F (**63**), which further confirmed its structure.

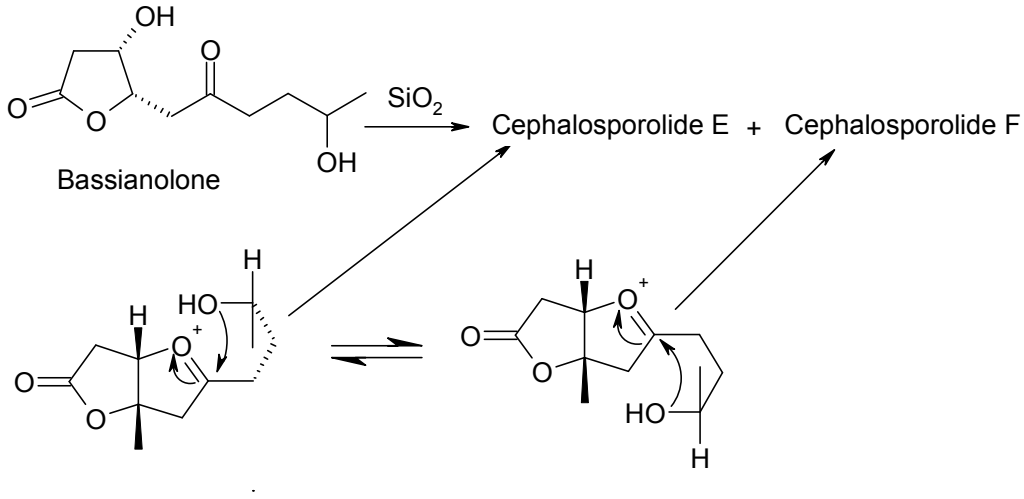
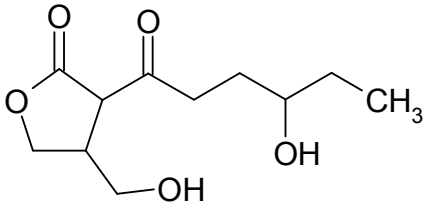


Figure 69. Silica-promoted spirocyclization of bassianolone (**65**) according to the literature.[100]



66

5.4 Marine-derived *Streptomyces* sp. B 7576

The marine *Streptomyces* sp. B7576 was selected for further investigations because of the very high activity against *Mucor miehei* (Tü 284) and weak activity against *Chlorella vulgaris and Chlorella sorokiniana.* The crude extract showed several bands during the screening by TLC. The most interesting band was not UV active and gave a green to brown colouration with anisaldehyde-sulphuric acid reagent and heating. The others exhibited UV absorption, among them one was middle polar and gave brown colour reaction with anisaldehyde/sulphuric acid. The crude extract of the water phase was first fractionated by silica gel column using a dichloromethane/methanol gradient to afford four fractions. These fractions were subjected to Sephadex LH-20 column chromatography employing elution with methanol to yield further sub fractions. Further purification of these sub fractions led to polypropylenglycol (Niax) (**67**) and 3,4-dihydroxy-3-methylpentan-2-one (**68**).

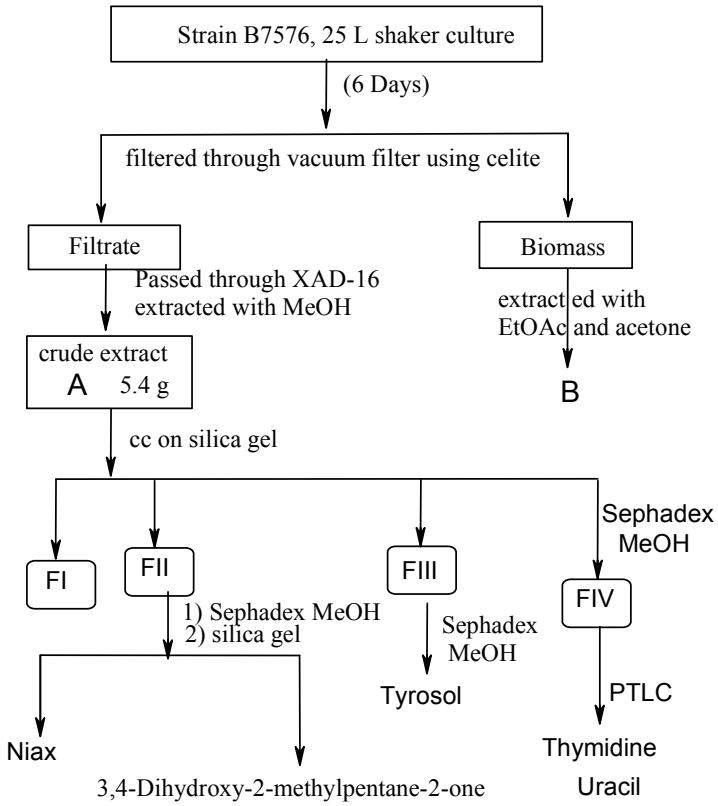
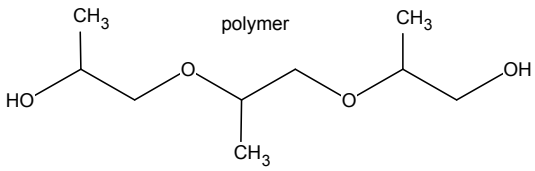


Figure 70. Work up scheme of marine derived *Streptomyces* sp. B 7576.

5.4.1 Niax (Polypropylenglycol)

Compound **67** was obtained as colourless oil, it displayed a non-UV active zone and gave pink colour by spraying with anisaldehyde/sulphuric acid reagent and heating. The ^{1}H NMR spectrum of compound **67** showed only two peaks in aliphatic re-

gion, one doublet signal at δ 1.10 for many CH_3 groups and a multiplet at δ 3.51 for many oxygenated methine and methylene groups. EIMS revealed a polymer of $-CH(CH_3)CH_2OH$ units by the continuous loss of $[M - 59]^+$ fragments. A search in AntiBase using the above spectroscopic data, the isolated compound was assigned as Niax (**67**). It is often used as antifoaming agent in fermentor cultures, however, was never applied in our shaker fermentations; we realized here and with other strains that it is also a natural product.



67

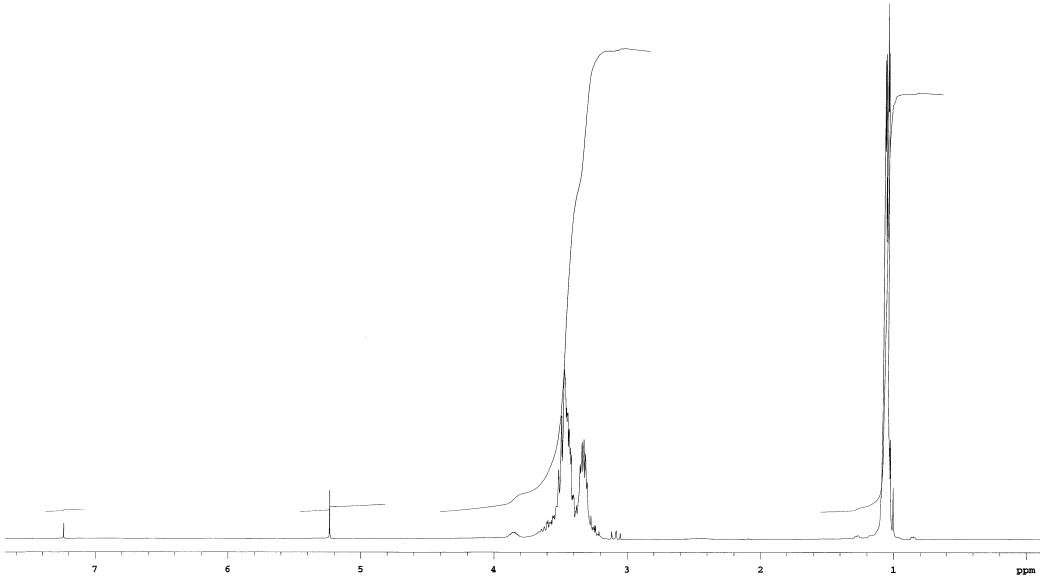


Figure 71. ^{1}H NMR spectrum (CD_3OD, 300 MHz) of Niax (**67**).

5.4.2 3,4-Dihydroxy-3-methylpentan-2-one

TLC of fraction II (Figure 70) exhibited a nonpolar compound which gave a green-brown band with anisaldehyde/sulphuric acid and heating. This fraction was chromatographed on Sephadex LH-20, followed by silica gel column using a dichloromethane/methanol gradient to afford compound **68**.

From the ^{1}H NMR spectrum, it was clear that a mixture of two diastereomers with ratio of 3:1 was present. The ^{1}H NMR spectrum for the first diastereomer (I) displayed a quartet at δ 4.04, a methyl singlet attached to a sp^2 carbon at δ 2.29 and a methyl singlet connected with sp^3 carbon at δ 1.26. Additionally, a methyl doublet at δ 1.25 was observed. The ^{1}H NMR spectrum of the second diastereomer (II) exhibited a

quartet at δ 3.87, a methyl singlet attached with sp^2 carbon at δ 2.25, a methyl singlet connected with sp^3 carbon at δ 1.42 and one methyl doublet at δ 1.08.

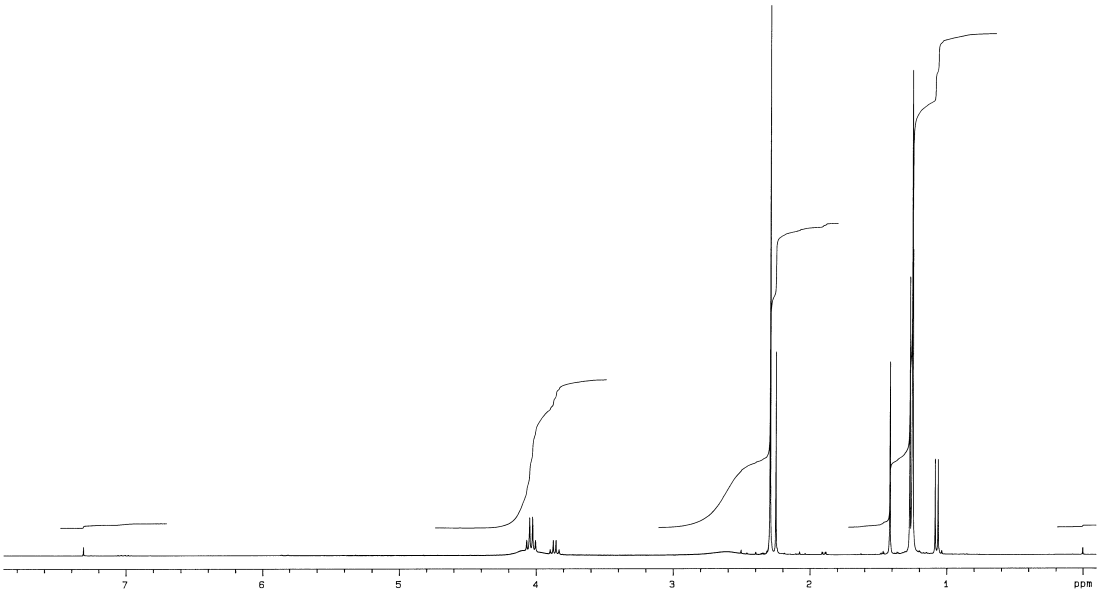


Figure 72. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3,4-dihydroxy-3-methylpentan-2-on (**68**).

The ^{13}C NMR spectrum showed a pair of signals at 211.7 (I) and 211.4 (II) for two ketone carbonyls, δ 81.4 (II) and 81.2 (I) for two oxygenated quaternary carbons, δ 71.2 (II) and 70.9 (I) for two methine carbons, δ 25.1 (II) and 23.9 (I) for two methyl signals, δ 21.7 (II) and 21.4 (I) for two methyl signals respectively. Finally, there were two methyl signals at δ 17.7 (II) and 16.7 (I).

The molecular weight was deduced from the DCI mass spectrum, which showed a molecular ion peak at *m/z* 150.2 ($[M + NH_4]^+$, 100%) and 167.2 ($[M + NH_3 + NH_4]^+$, 12%) corresponding to a molecular weight of 132 Dalton. The H,H COSY showed strong ^3J correlation between the methyl protons at 1.26 (I), 1.08 (II) and the oxygenated methines at δ 4.04 (I), 3.87 (II). From the H,H COSY and ^{13}C NMR spectra the following fragments could be construct (Figure 73). With the aid of HMBC correlations theses fragments were connected to give the structures of compounds **68a,b**. The diastereomers were further confirmed by comparison of their spectroscopic data with authentic spectra.[101,102]

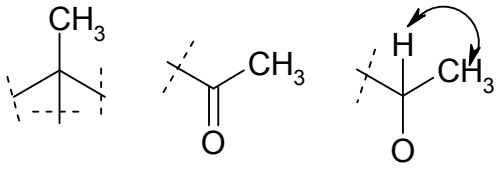


Figure 73. Fragments constructed from H,H COSY and ^{13}C NMR spectra.

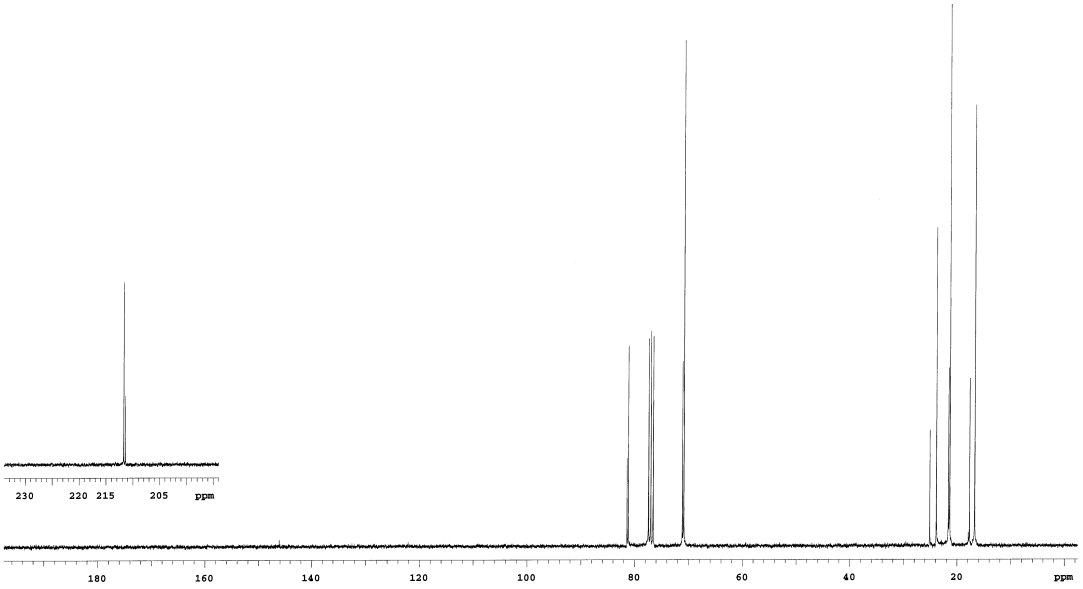
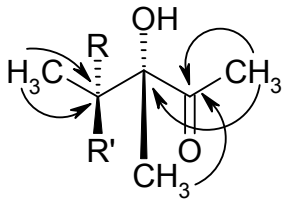


Figure. ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 2,3-dihydroxy-3-methylpentan-2-one (**68**).



68a R = H, R' = OH **68b** R = OH, R' = H

Compounds **68a,b** were isolated by Mi-Young and coworkers in 2006 from quickly fermented soy-based foods and they have antioxidative activity.[103]

5.5 Marine derived *Streptomyces* sp. B 8073

The marine *Streptomyces* sp. B 8073 formed white mycelial colonies after incubation on M_2^+ agar medium for 6 days at 28 °C. The antimicrobial assay of this strain showed a high activity against *Staphylococcus aureus, Escherichia coli,* and *Bacillus*

subtilis. It also showed high activity against the fungus *Mucor miehei* and moderate cytotoxic activity against *Artemia salina*. The chemical screening using TLC showed highly blue fluorescent regions, which stained to yellow and brown with anisaldehyde/sulphuric acid.

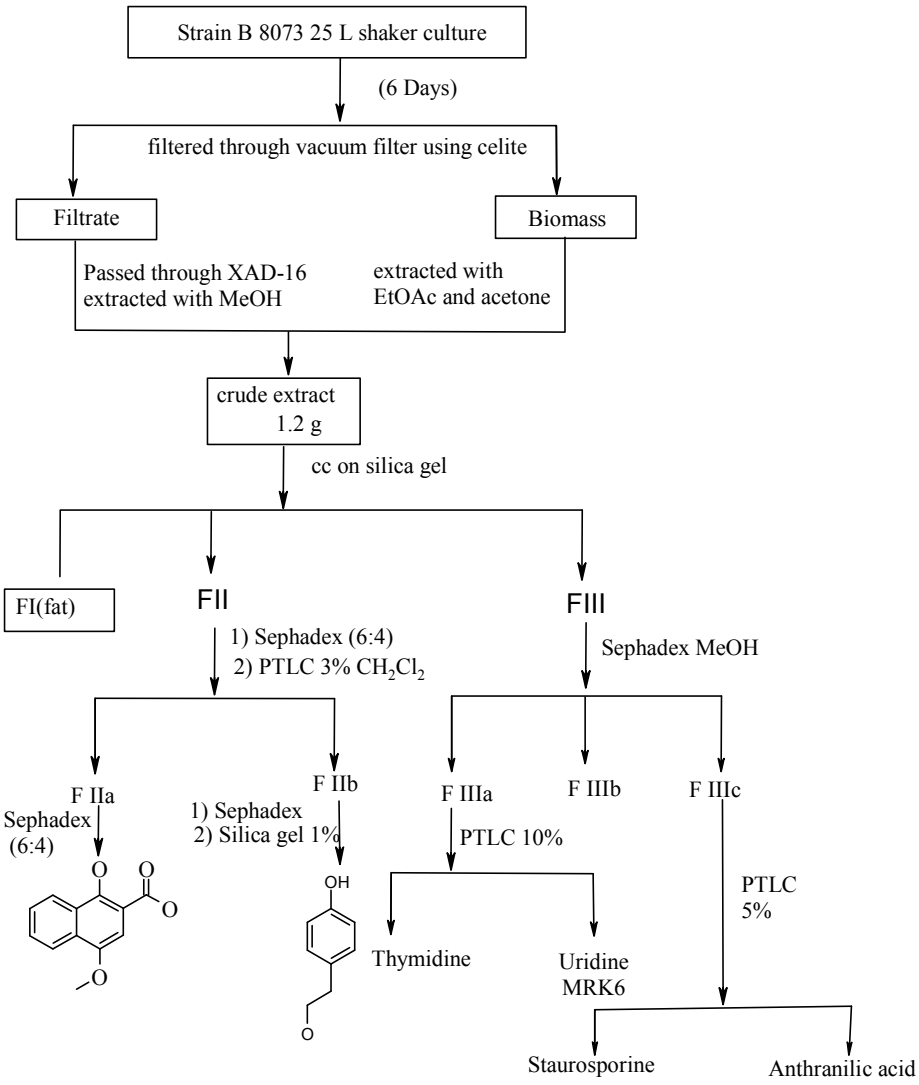
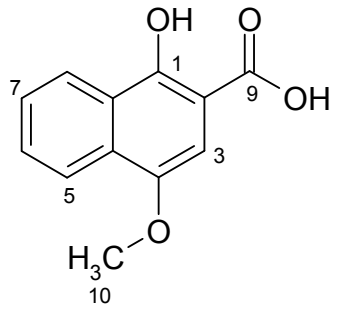


Figure 74. Work up scheme of the strain *Streptomyces* sp. B 8073.

5.5.1 1-Hydroxy-4-methoxy-2-naphthoic acid

Compound **69** was obtained as an amorphous powder with the molecular weight 218 determined by ESIMS, corresponding to the molecular formula $C_{12}H_{10}O_4$. The ^1H NMR spectrum of **69** showed five signals in the aromatic region, two of them were observed as doublet of doublet at δ 8.25 and 8.11, while another two protons came together in the same position at δ 7.48, the last one was displayed at δ 7.29 as singlet. In addition, an oxygen-bearing methyl at δ 3.95 was present. A search in AntiBase using the above spectroscopic data as well as the molecular weight gave coincidence

with 1-hydroxy-4-methoxy-2-naphthoic acid (**69**). This was further confirmed by comparing with authentic spectra as well as literature data. The herbicidal **69** was previously isolated from *Streptosporangium cinnabarinum* ATCC 31213.[104]



69

Table 7: Comparison of ^{1}H NMR (300 MHz) chemical shift of 1-Hydroxy-4-methoxy-2-naphthoic acid (**69**) with reference data.[104]

Proton No	$^{1}H^{a}$ (Int., mult, *J* [Hz]) lit.[104]	$^{1}H^{a}$ (Int., mult, *J* [Hz])
3	7.19 (s)	7.29 (s)
5	8.18 (1H, d, 8.1)	8.11 (1H, dd, 8.1, 2.0)
6	7.62 (1H, ddd, 6.9, 1.2)	7.48 (2H, ddd, 6.9,1.2)
7	7.56(1H, ddd, 8.0, 1.2)	7.48 (1H, ddd, 6.9, 1.2)
8	8.33 (d, 1H, 8.0)	8.25 (1H, dd, 8.0, 2.0)
10-OCH_3	3.96 (3H, s)	3.95 (3H, s)

(^{a} MeOH)

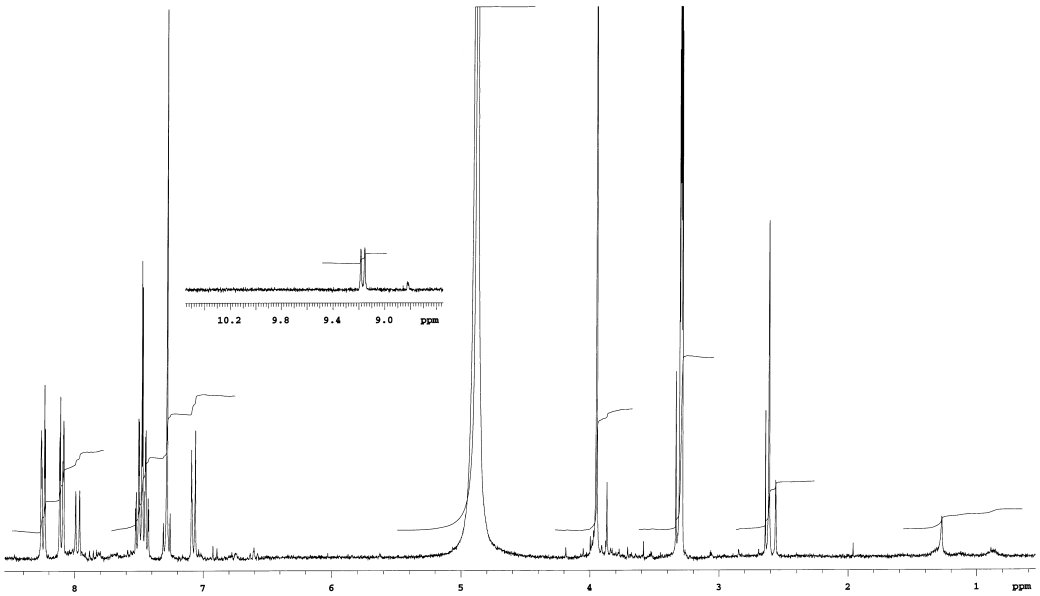


Figure 75. ^{1}H NMR spectrum (300 MHz, CD_3OD) of 1-Hydroxy-4-methoxy-2-naphthoic acid (**69**).

5.5.2 Staurosporin

Compound **70** was obtained as yellow solid from subfraction IIIc (Figure 74) by PTLC. It showed strong blue fluorescence (366 nm) and an UV absorbing band at 256 nm and stained to brown with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of **70** exhibited in the aromatic region a doublet at δ 9.24 (H-4), triplet for two protons at δ 7.98 (H-8) and (H-11) and a doublet at δ 7.57 (H-1) corresponding to 1,2-disubstituted benzene ring. Furthermore, two methine proton signals attached to heteroatom N/O were observed at 4.07 and 3.28 as well as to an anomeric proton at 6.70 (H-6'). Finally in the aliphatic region, three methyl singlets at δ 3.30 for methoxy, 2.31 and 1.53 probably attached to sp^2 carbon or nitrogen were present.

The ESI mass spectrum displayed $[2\ M + H]^+$ and $[M + H]^+$ ion peaks at *m/z* 933 and 466, respectively, consistent with the molecular formula $C_{28}H_{26}N_4O_3$. A search in AntiBase using the spectroscopic data as well as molecular weight gave coincidence with staurosporine (**70**). This was further confirmed by comparing with authentic spectra as well as literature data.[105]

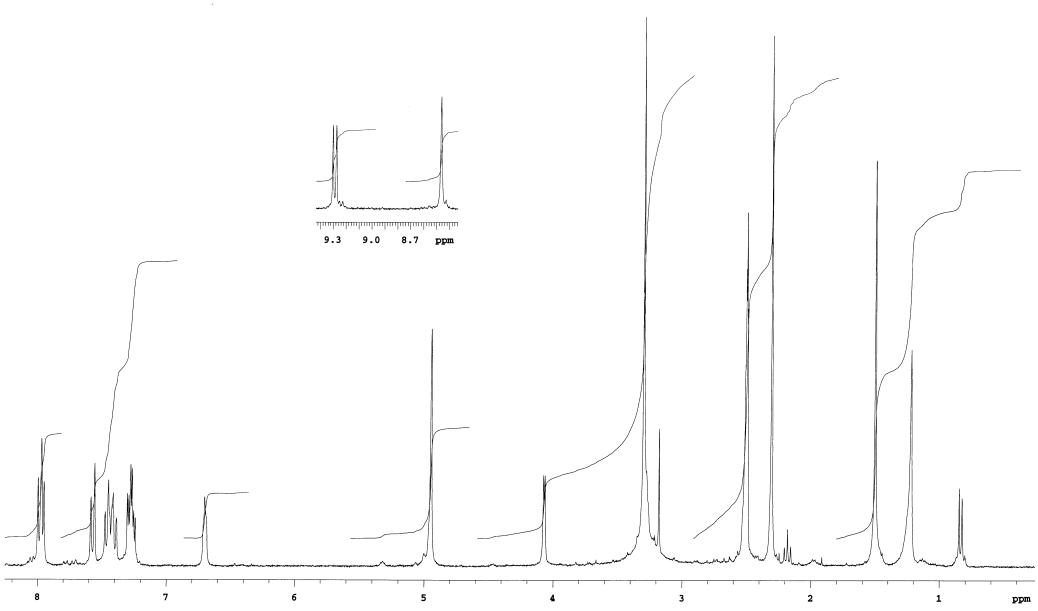
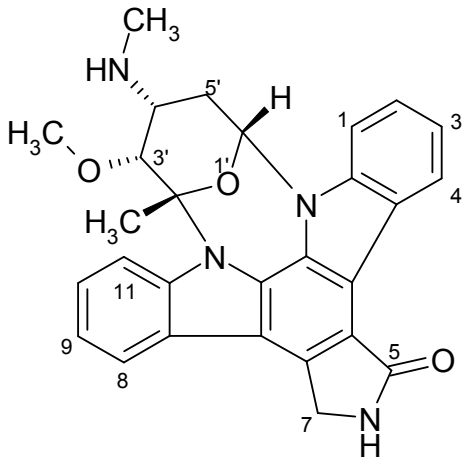


Figure 76. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of staurosporin (**70**).



70

Staurosporine (**70**) is an indolo[2,3-a]carbazole alkaloid and was firstly isolated from *Streptomyces staurosporeus* Awaya (AM-2282)[106] and later from several other actinomycetes. Staurosporine (**70**) possesses biological activity against fungi and yeasts but has no significant activity effects against bacteria; it also revealed strong antihypertensive activity and distinct antitumor activity *in vitro*.[107] Most interesting is its potent inhibition of protein kinase C and the formation of blood clots.[108] The aglycone moiety of staurosporine was found to be responsible for the biological activity, which indicated that other indolo[2,3-a]carbazole derivatives might also possess significant biological activity.

5.6 Marine *Streptomyces* sp. B8013

The crude extract of the marine *Streptomyces* sp. B8013 exhibited moderate activity against *Mucor miehei* (Tü284), *Candida albicans and Staphylococcus aureus*. TLC analysis of the extract showed three different coloured zones: orange, violet, and green with anisaldehyde/sulphuric acid.

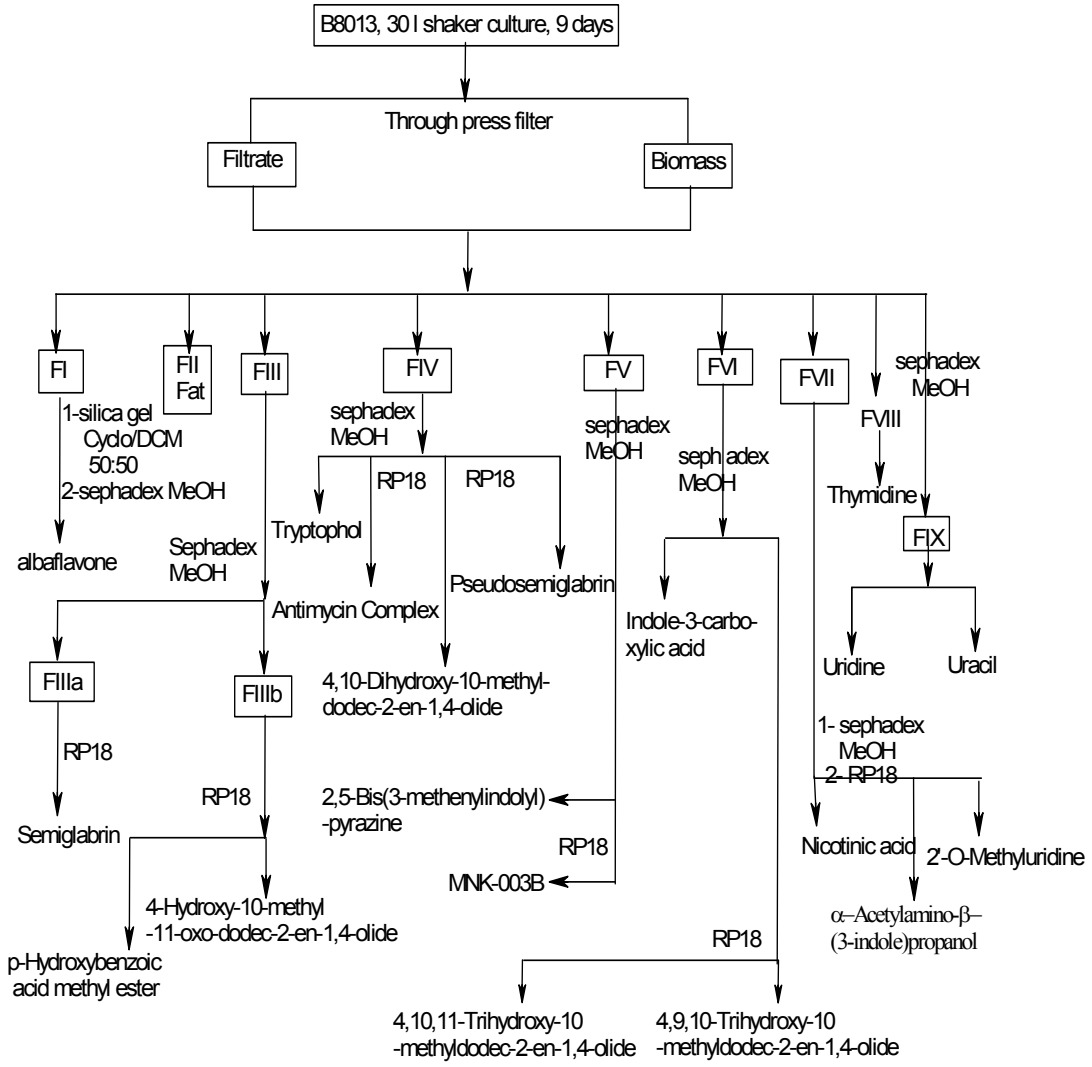


Figure 77. Work-up scheme Marine *Streptomyces* sp. of B8013.

5.6.1 Albaflavenone

Fraction I showed non-polar and non-UV active zone, which turned to orange with anisaldehyde/sulphuric acid and heating. Further purification of this fraction using silica gel column and dichloromethane, then Sephadex LH-20 led to oily colourless albaflavenone (**71**). The ^{1}H NMR spectrum of **71** displayed neither aromatic, olefinic nor oxygenated protons. An ABX system at δ 2.37 (Ha-3) and 2.15 (Hb-3) corresponding to methylene protons connected to sp^2 carbon and one singlet at δ 2.06 (CH_3-13) attributed to allylic methyl group, were observed in the aliphatic region. Complex multiplet signals of methylene and methine protons between δ 1.90-1.30 were present. In addition, three methyls were observed at δ 1.03 (H-12), 1.09 (H-15) and 1.12 (H-14) respectively.

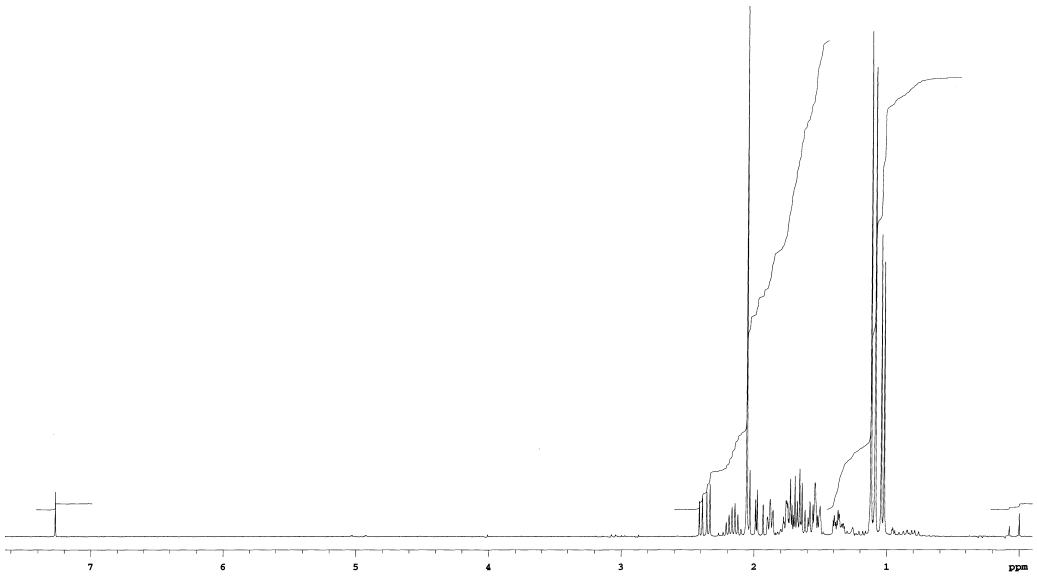


Figure 78. ^{1}H NMR spectrum (300 MHz) of Albaflavenone (**71**) in $CDCl_3$.

The ^{13}C spectrum exhibited 15 carbon signals, which could be attributed to a sesquiterpene. The signal at δ 207.3 was interpreted as carbonyl of a ketone or aldehyde. The two quaternary carbons at δ 153.0 and 138.9 represented a carbon double bond fragment in conjugation with a carbonyl group, while the signal at δ 28.4, 24.5,14.2 and 13.0 accounted for four methyl groups. The ESI mass spectrum indicated the *pseudo*-molecular peak at *m/z* 459 $[2\ M+\ Na]^+$ and 241 $[M + Na]^+$ which fixed the molecular weight of 218 Dalton.

A search in AntiBase supported by ^{1}H, ^{13}C NMR, and MS spectroscopic data led to albaflavenone (**71**) as a possible structure. This was further confirmed by comparison of its spectroscopic data with the literature.[109] Albaflavenone is an α,β-unsaturated sesquiterpene with a zizaene skeleton. It was isolated for the first time by Gurtler *et al.* in 1994 from morphologically novel, highly odorous *Streptomyces* species, which was identified as belonging to the species group *S. albidolflavus*. Albaflavenone had weak activity against *Bacillus subtilis.*

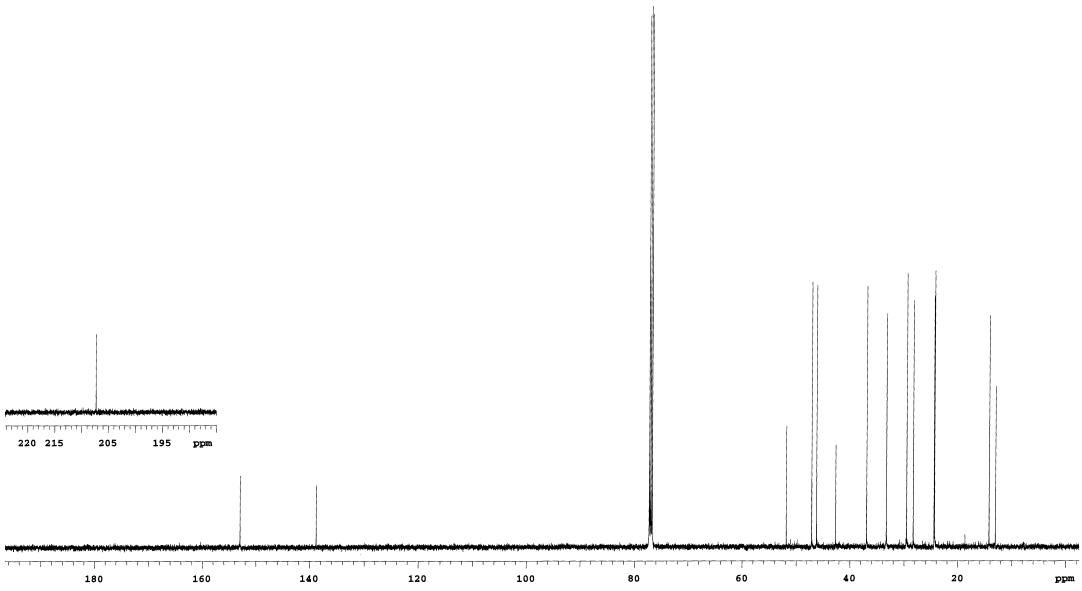
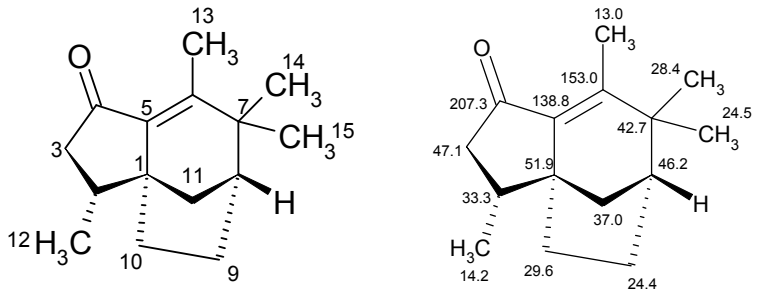


Figure 79. ^{13}C NMR spectrum (125 MHz) of Albaflavenone (**71**) in $CDCl_3$.



71

Table 8. Comparison of ^{13}C NMR chemical shifts of Albaflavenone (**71**) with the literature data.[109]

position	Lit. (δc)	Exp (δc)	position	Lit. (δc)	Exp(δc)
1	51.8	51.9	9	24.4	24.4
2	33.3	33.3	10	26.6	29.6
3	47.4	47.1	11	37.0	37.0
4	207.4	207.3	12	14.2	14.2
5	138.8	138.8	13	13.0	13.0
6	153.0	153.0	14	28.3	28.4
7	42.7	42.7	15	24.5	24.5
8	46.2	46.2			

5.6.2 Pseudosemiglabrin

The chromatogram of fraction IV showed a blue fluorescence zone, which gave a blue-green colour with anisaldehyde/sulphuric acid on heating. Fraction IV was applied to Sephadex LH-20, then a RP-18 column to afford compound **72** as white solid.

The ^{1}H NMR spectrum showed several signals with a total intensity of 20 protons. Two *o*-coupled aromatic protons centred at δ 8.18 (d, H-5, ^{3}J = 8.6 Hz) and 6.96 (d, H-6, ^{3}J = 8.6) Hz, 1H singlet at δ 6.78, which was a typical signal of flavones. Three aromatic signals with the intensity of five protons were indicative for the presence of a mono-substituted benzene ring (ring B). Moreover, three methine protons at δ 6.52 (d, H-2") possibly dioxygenated, 5.58 (d, H-3"') oxygenated and 4.63 (d, H-3") connected to sp^2 carbon appeared. In the upfield region, methyl groups were observed at δ 1.40 and 1.15 assigned to H-4"' and H-5"', respectively, adjacent to an oxygenated carbon. One upfield acetyl group was observed at δ 1.49 due to the ring current effect of the benzene ring (B).

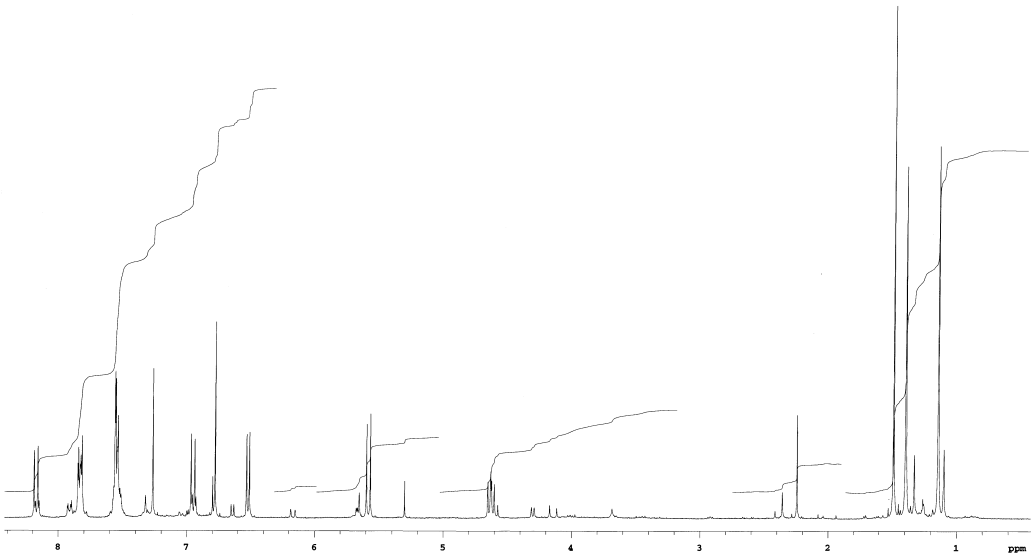


Figure 80. ^{1}H NMR spectrum (300 MHz) of pseudosemiglabrin (**72**) in CD_3OD.

The ^{13}C NMR spectrum showed the presence of one ketone carbonyl at δ 177.7, three oxygenated sp^2 carbon signals at δ 164.6 (C-7), 153.8 (C-8) and 162.7(H-2), as well as another 11 sp^2 signals between 107.5-131.8 to establish altogether a flavone moiety (ring A, B and C). Three methine carbon signals were assigned to an acetal carbon at δ 111.7, (C-2"), an oxymethine at 77.0 (C-3"') and another one connected to an sp^2 carbon at δ 47.9 (C-3"). In addition, there were three methyl signals assigned to C-4"' (δ 27.6), C-5"' (δ 23.2), and an acetyl group (δ 20.3).

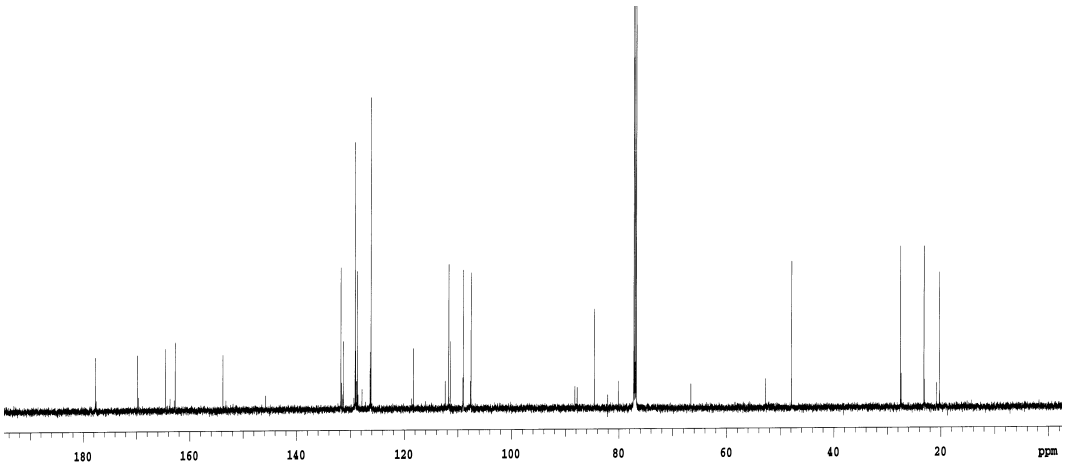
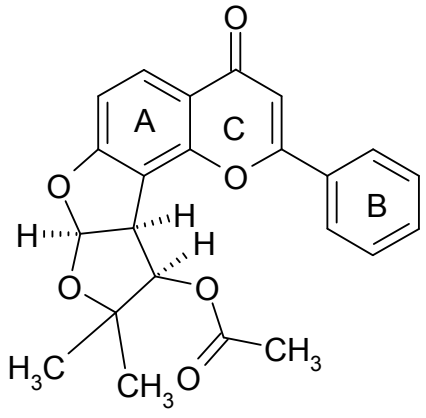
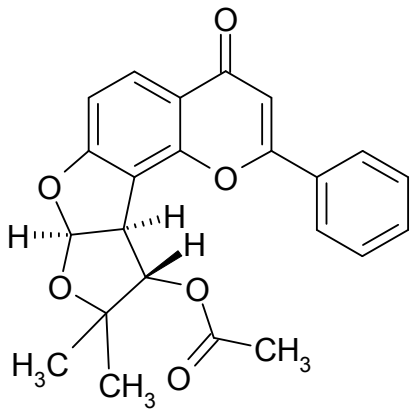


Figure 81. ^{13}C NMR spectrum (150 MHz) of pseudosemiglabrin (**72**) in CD_3OD.

The molecular formula was deduced as $C_{23}H_{20}O_6$ on the basis of the HRESIMS data with an exact mass of the $[M + H]^+$ ion at *m/z* 393.13326. The EI mass spectrum of compound **72** exhibited a molecular ion peak at *m/z* 392 as well as to ion peak at *m/z* 332 due to the loss of acetic acid. Furthermore, there was a significant ion peak at *m/z* 102 matched with Ph-CH=CH^+, the fragment commonly displayed in the mass spectra of flavones with an unsubstituted B-ring.[110] A search in AntiBase gave no hits, which indicated that this compound was new from microorganism, while the Chemical Abstracts showed two possible structures, pseudosemiglabrin (**72**) and semiglabrin (**73**).



72



73

The H,H COSY spectra showed the presence of one mono-substituted benzene ring, as well as the methine proton at δ 4.63 showed ^3J correlation with those at δ 6.52 and 5.58. The partial structures derived from the ^1H NMR and H,H COSY are given below:

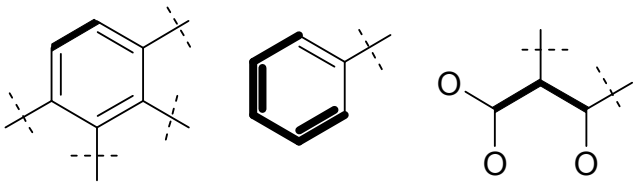


Figure 82. Fragments of pseudosemiglabrin (**72**).

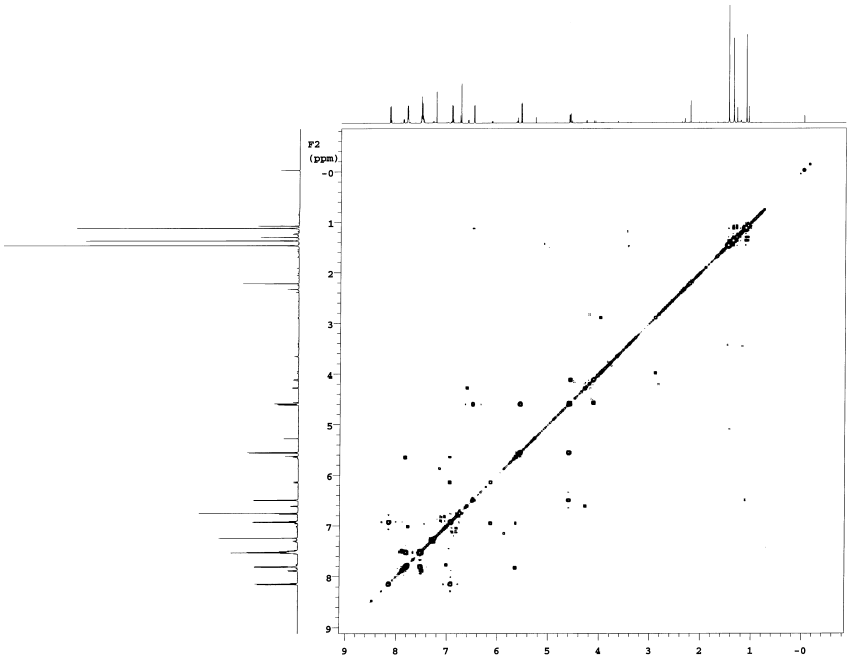


Figure 83. H,H COSY spectrum (600 MHz) of pseudosemiglabrin (**72**) in CD_3OD.

In the HMBC spectrum the proton H-5 (8.18) showed a three-bond correlation with the carbonyl CO-4 (177.7), C_q-9 (153.8) and C_q-7 (164.6). The *gem*-dimethyl H-4''' (27.6) and H-5''' (23.2) correlated to C_q-2''' (84.6). The anomeric proton H-2'' exhibited a three-bond correlation with C_q-7, C_q-8 (111.4), CH-3''' and C_q-2'''. The detailed interpretation of the H,H COSY, HMQC and HMBC correlations, unambiguously confirmed the structure of pseudosemiglabrin (**72**). The relative configuration was derived from the NOE of H-3'' with H-2'' and H-3'''.

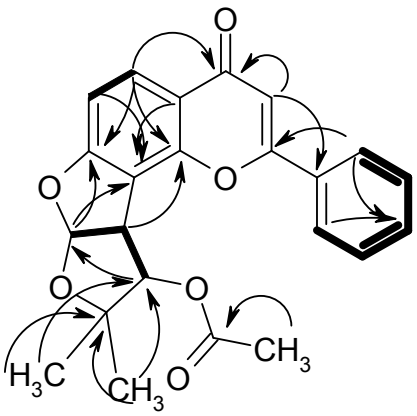


Figure 84. H,H COSY (▬) and selected HMBC (→) correlations of pseudosemiglabrin (**72**).

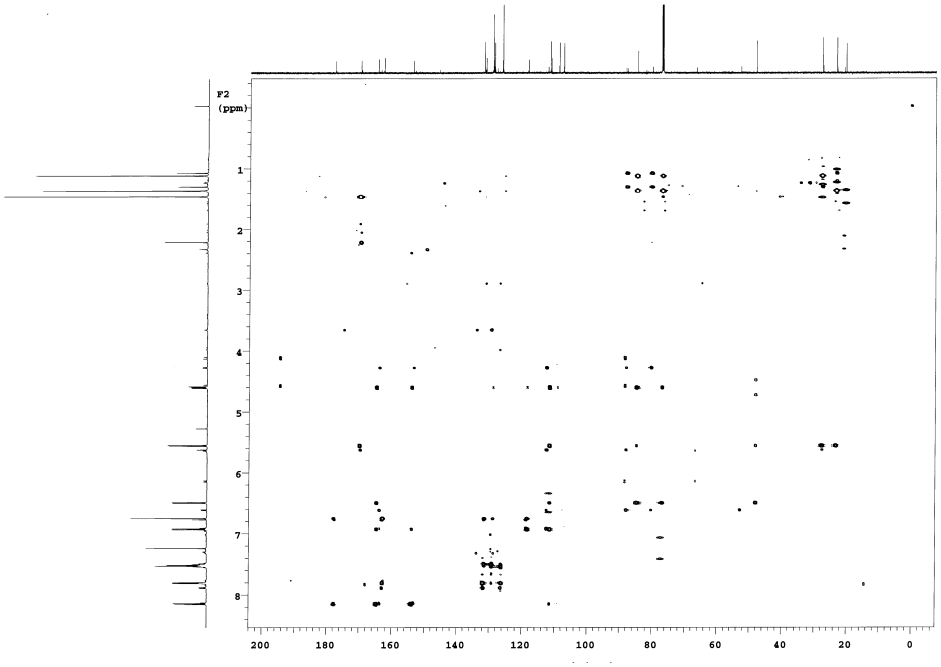


Figure 85. HMBC spectrum (600 MHz) of pseudosemiglabrin (**72**) in CD_3OD.

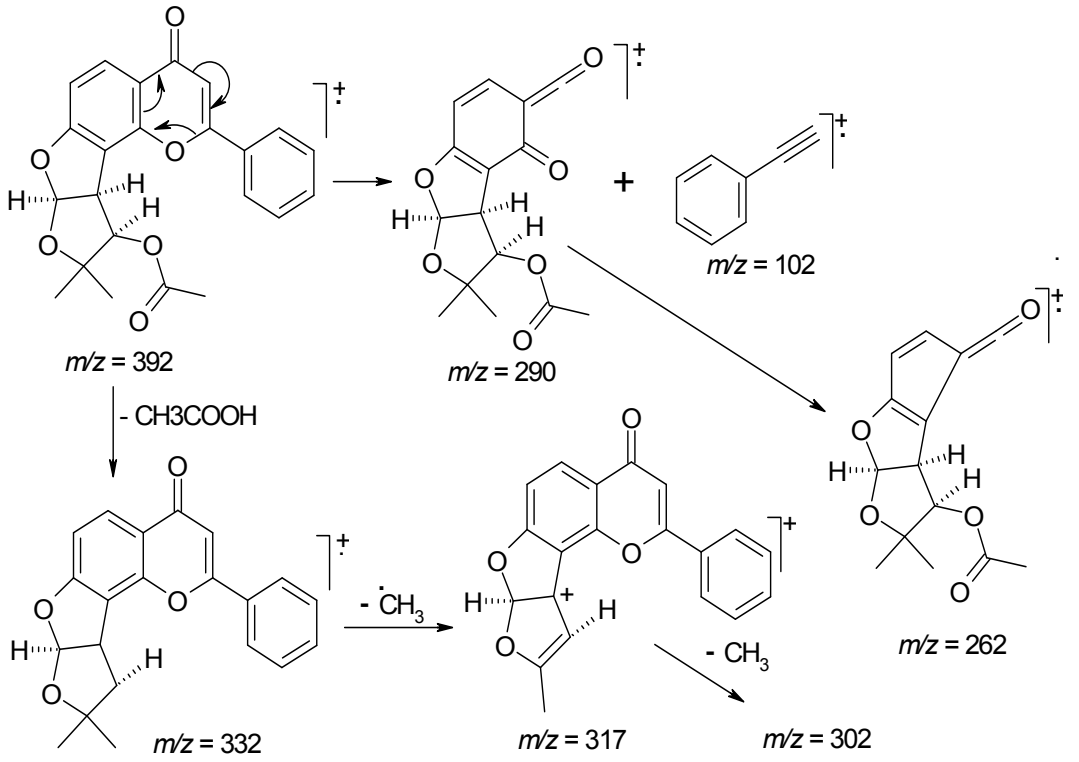
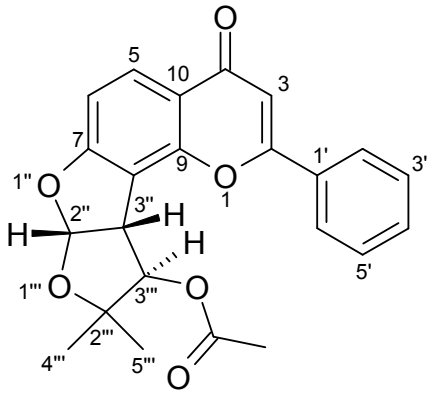


Figure 86. EI mass fragmentation pattern of pseudosemiglabrin (**72**).

5.6.3 Semiglabrin

Compound **73** showed the same chemical and physical properties as pseudosemiglabrin (**72**). Also all spectroscopic data of this compound were identical with those of pseudosemiglabrin (**72**). As in ^{1}H NMR and ^{13}C NMR spectra of pseudosemiglabrin, the spectra of semiglabrin showed 20 protons and 23 carbon signals, respectively, and with the high resolution mass, the formula $C_{23}H_{20}O_6$ resulted. There was only a difference in the chemical shift value of the acetyl group between semiglabrin and pseudosemiglabrin.



73

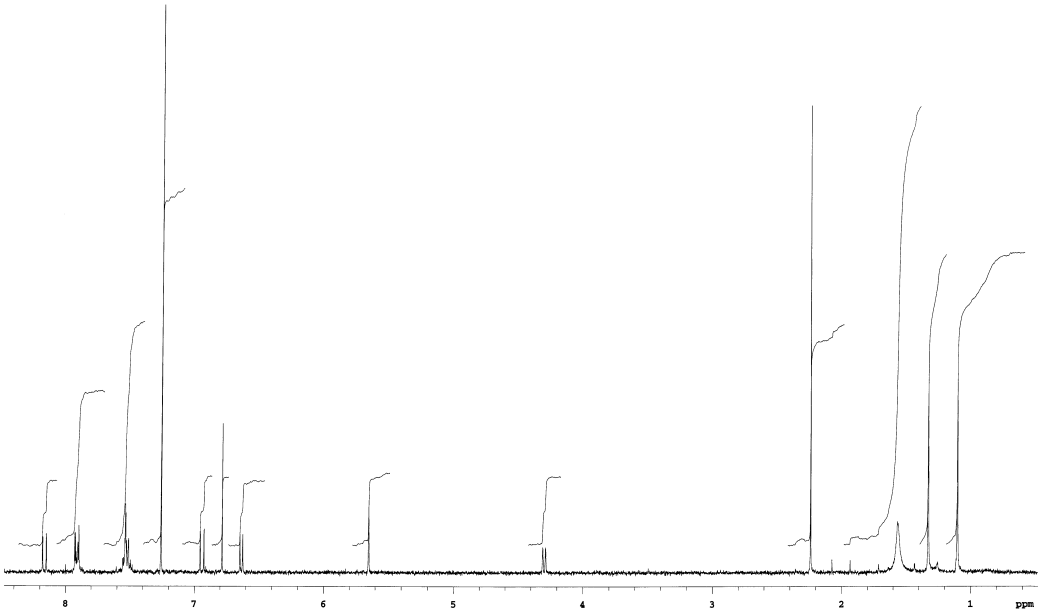


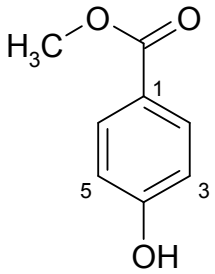
Figure 87. ^{1}H NMR spectrum (600 MHz) of semiglabrin (**73**) in $CDCl_3$.

The structures of compounds **72** and **73** were confirmed by comparison of their spectroscopic data with the literature values.[111,112] Pseudosemiglabrin (**72**) and semiglabrin (**73**) have been isolated from several *Tephrosia* species, including *Tephrosia apollinea*,[113-115] *T. semiglabra*[116], *Tephrosia nubica*[117], *Tephrosia pupurea*[112,118] and *T. hokeriana*.[119] Pseudosemiglabrin (**72**) inhibited human platelet aggregation *in vitro*.

5.6.4 *p*-Hydroxybenzoic acid methyl ester

The ^{1}H NMR spectrum *p*-hydroxybenzoic acid methyl ester (**74**) showed two *o*-coupled aromatic signals at δ 7.92 (d, H-2,6) and 6.84 (d, H-3,5) each with integration of 2H which pointed to AA',BB' pattern. These patterns are characteristics of a 1,4-disubstituted benzene ring. The upfield shift of the latter signal is the evidence of electron donating group on the aromatic ring. The spectrum showed also a methoxy signal at δ 3.83.

The molecular weight of compound 74 was determined as 152 Dalton by EIMS. In addition, a base peak was obtained at *m/z* 121, as a result from the expulsion of OCH_3. A search in AntiBase using the ^{1}H NMR data as well as the molecular weight revealed *p*-hydroxybenzoic acid methyl ester, which was further confirmed by comparison with an authentic spectrum.[120]



74

5.6.5 Antimycin A-Complex

Chromatography of fraction IV (Figure 77) on Sephadex LH-20 gave a colourless oil. TLC of compound **75** displayed a spot with strong fluorescence at 366 nm, which showed no colouration on spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **75** displayed three D_2O exchangeable protons at δ 12.66 (s), 7.98 (br s, NH), and 7.17 (d, NH). Furthermore, a doublet at δ 8.52 (^{3}J = 1.7 Hz) was assigned to an aldehydic proton, signals for three aromatic protons in 1,2,3-position at δ 8.56 (d), 7.24 (d) and 6.90 (t), four oxymethines at δ 5.78 (quintet, H-4), 5.38 (t, H-3), 5.10 (m, H-8) and 4.99 (m, H-9) were observed. In the aliphatic region three complex multiplets in the range of δ 2.60-1.40, 1.40-1.00 and 1.00-080 were present. The (+)-ESI mass spectrum showed four $[M + Na]^{+}$ ion peaks corresponding to the molecular weights 562, 548, 534, 520 which confirmed the mixture, which had already been expected from the complexity of the spectrum and the ratio of the integrals. A search in AntiBase with these data led to the identification of compound **75** as antimycin A-complex.

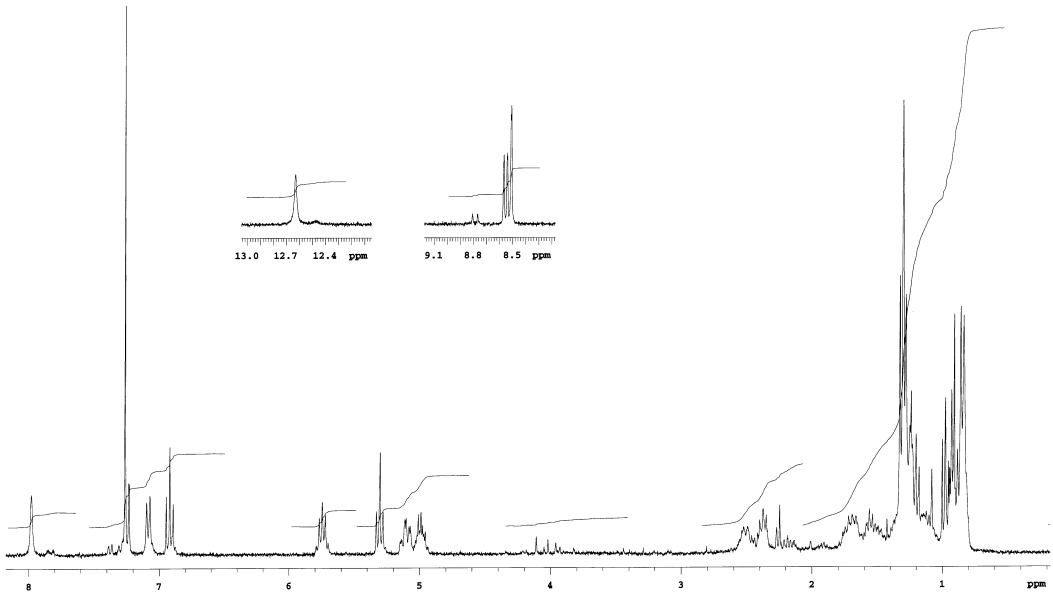
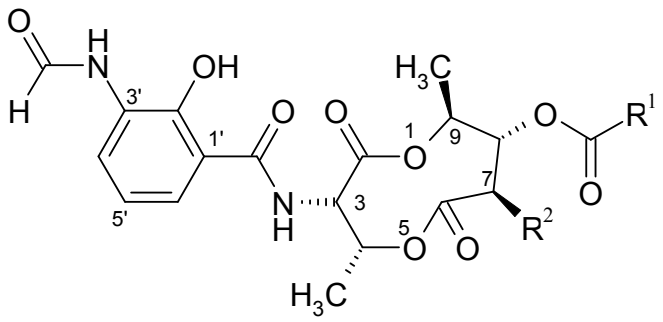


Figure 88. ^{1}H NMR spectrum (300 MHz) of antimycin A-complex (**75**) in $CDCl_3$



75

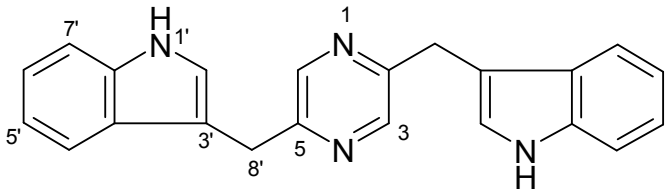
Antimycin (**75**)	R^1	R^2
A_1	$CH(CH_3)CH_2CH_3$	$(CH_2)_5CH_3$
A_2	$CH_2CH_2CH_3$	$(CH_2)_5CH_3$
A_3	$CH(CH_3)CH_2CH_3$	$CH_2CH_2CH_2CH_3$
A_4	$CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$
A_7	$CH_2CH_2CH_2CH_3$	$(CH_2)_2CH(CH_3)_2$
A_8	$CH_2CH(CH_3)_2$	$(CH_2)_2CH(CH_3)_2$
A_9	$CH_2(CH_2)_2CH_3$	Ph

The antimycin family of antibiotics shares a common structural feature; a nine-membered dilactone ring, the 3-formamidosalicylamide unit and two alkyl side chains of varying length. They were isolated from a *Streptomyces* sp. for the first time in 1949;[121] the structure of antimycin A_1 was completed in 1961[122,123] and its absolute configuration solved in 1972 by Kinoshita.[124] These compounds possess various biological properties including ichthyotoxic[125], insecticidal,[125] antifungal,[126-128] anti-cancer[129] activities and inhibit ATP-citrate lyase against the substrate magnesium citrate.[130] Recently antimycin A9 isolated from *Streptomyces* sp. K01-0031 showed more potent nematocidal and insecticidal activities against *Caenorhabditis elegans* and *Artemia salina* than the other known antimycins.[131] Recently another seven antimycin antibiotic were isolated from *Streptomyces* spp. SPA-10191 and SPA-8893.[132] Due to their outstanding bioactivities, antimycins have attracted much interest from synthetic organic chemist.[133,134]

5.6.6 2,5-Bis(3-indolylmethyl)pyrazine

A faint yellow band of compound **76** was observed in the fraction FV (Figure 77). This band showed UV absorption at 254 and fluorescence at 366 nm and turned to pink with Ehrlich's reagent (and later to faint brown). The band was purified by Sephadex LH-20 to give **76** as a faint yellow solid.

The ^{1}H NMR spectrum of **76** showed six aromatic proton signals between δ 8.38 and 6.94, four of them were observed at δ 7.38 (d, 7.9 Hz), 7.07 (t, 8.1 Hz), 7.33 (d, 7.9 Hz), 6.94 (t, 7.8) belonging to a 1,2-disubstituted benzene ring. The downfield signals at δ 8.38 suggested an electron-deficient heteroaromatic ring. One singlet signal at δ 7.09 was indicative for H-2 of an indole system. Finally, the ^{1}H NMR spectrum displayed one methylene singlet at δ 4.25 could be oxygenated or connected to two sp^2 carbons. This indole skeleton was proved by the pink colour reaction with Ehrlich's reagent and the brown colour with anisaldehyde-sulphuric acid and heating. The ESIHRMS data of **76** led to the molecular formula $C_{22}H_{18}N_4$ with 16 double bond equivalents. For further confirmation the spectroscopic data were compared with those previously published. Compound **76** has been isolated from bacterial strain *Cytophaga* sp. AM 13.1, it showed no activity against bacteria, fungi and algae.[135]



76

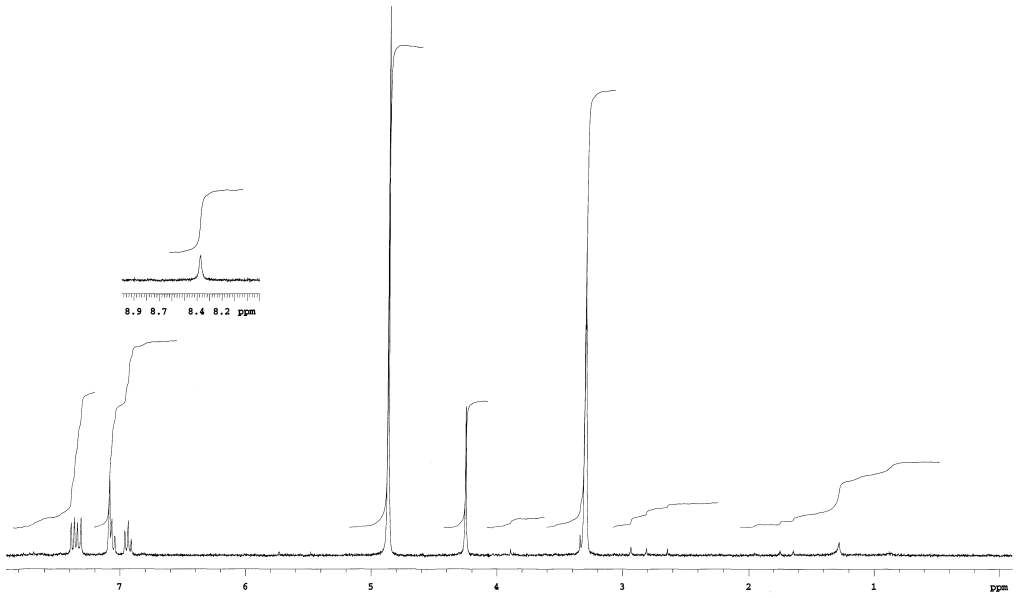


Figure 89. ^{1}H NMR spectrum (300 MHz) of 2,5-bis(3-indolylmethyl)pyrazine in CD_3OD (**76**).

5.6.7 MNK-003B

The separation of fraction FV using Sephadex LH-20 and finally RP18 led to the isolation of an oily colourless pure compound **77**. It was not UV-active and gave a violet colour reaction on spraying with anisaldehyde/sulphuric acid, which turned to blue with the time. The ^{1}H NMR spectrum showed in the sp^2 region only two 1H signals at δ 7.46 (dd, J = 5.7, 1.5 Hz) and 6.12 (dd, J = 5.7, 1.5 Hz). From the small coupling constants (5.7 Hz) it was clear that compound **77** contained a small ring like a five membered heterocycle. Moreover, a 1H signal at δ 5.03 (m) assigned to a methine proton bearing an oxygen atom, overlapping multiplets due to five methylenes between δ 1.90-1.30 and a 6H singlet at δ 1.22 attributed to a *gem*-dimethyl group were observed.

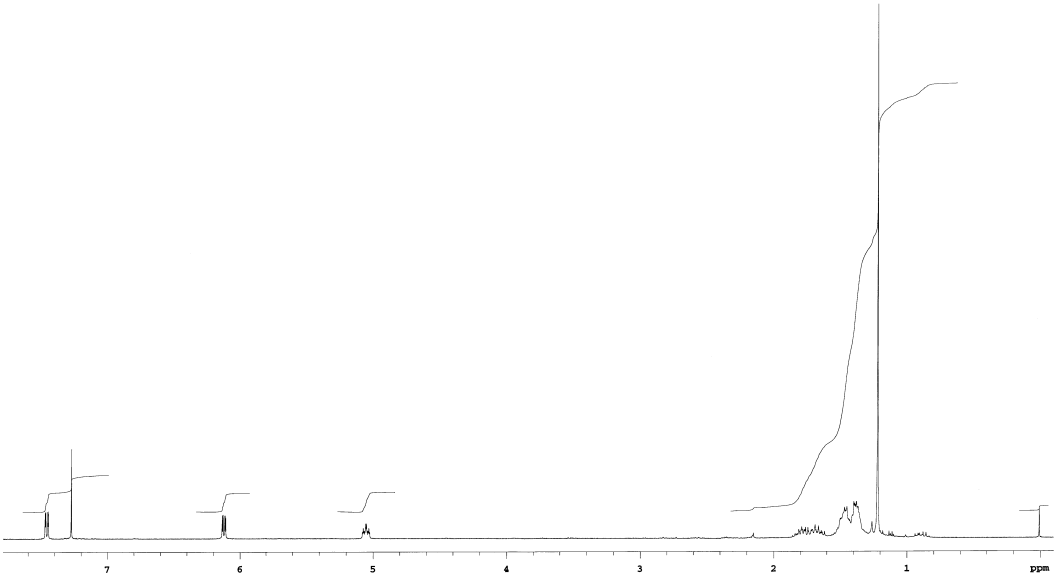
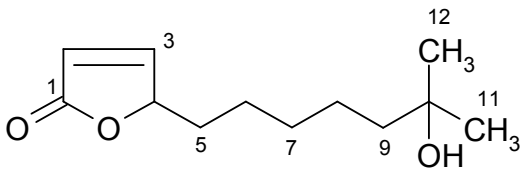


Figure 90. ^{1}H NMR spectrum on MKN-003B (300 MHz) in $CDCl_3$ (**77**).

The low-resolution ESIMS spectrum of compound **77** showed the *pseudo*-molecular ion peak at *m*/*z* 235 $[M + Na]^+$, which fixed the mass as 212 Dalton. A search in AntiBase and Scifinder using the above spectroscopic data led to MNK-003B (**77**) as the possible structure, which was further confirmed by comparison with an authentic spectrum and the literature. Compound **77** was isolated by Ki Woong *et al*. in 2001 from a marine-derived bacterium.[136]

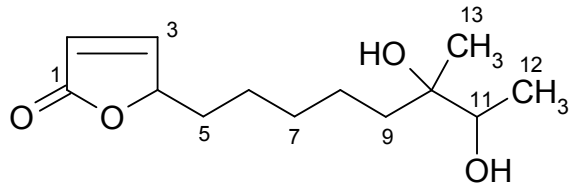


77

5.6.8 4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide

Purification of sub fraction VI on Sephadex LH-20 followed by RP-18 delivered oily colourless compound **78**. Compound **78** showed on TLC the same characteristics as **77** on spraying with anisaldehyde/sulphuric acid and heating. Also the ^{1}H NMR spectrum showed very close similarity to that of **77**. Two oxymethine signals at δ 5.14 (m, H-4) and 3.55 (q, H-11) were present instead of one in **77**. Finally the two *gem*-dimethyl groups in **77** were replaced by one methyl doublet at δ 1.07 for a $CH_3CH(OH)$ group and one methyl single at δ 1.11.

The ESI mass spectra determined the molecular weight of **78** as 242 Dalton. Two *quasi*-molecular ion peaks were observed in the (+)-ESI mode at *m/z* 506 $[2\ M+ Na]^+$, and 265 $[M + H]^+$, while the (-)-ESI mode exhibited one *quasi*-molecular ion peak at *m/z* 241 ($[M - H]^-$), which corresponded to the molecular formula $C_{13}H_{22}O_4$. A literature search resulted in the identification of compound **78** as 4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide. The structure of **78** was further confirmed by comparing with the authentic spectra as well as literature data.[137] Compound **78** was isolated in our group by Fotso from marine-derived *Streptomyces* sp. Mei 35, and *Streptomyces* sp. LR4612.[137,138]



78

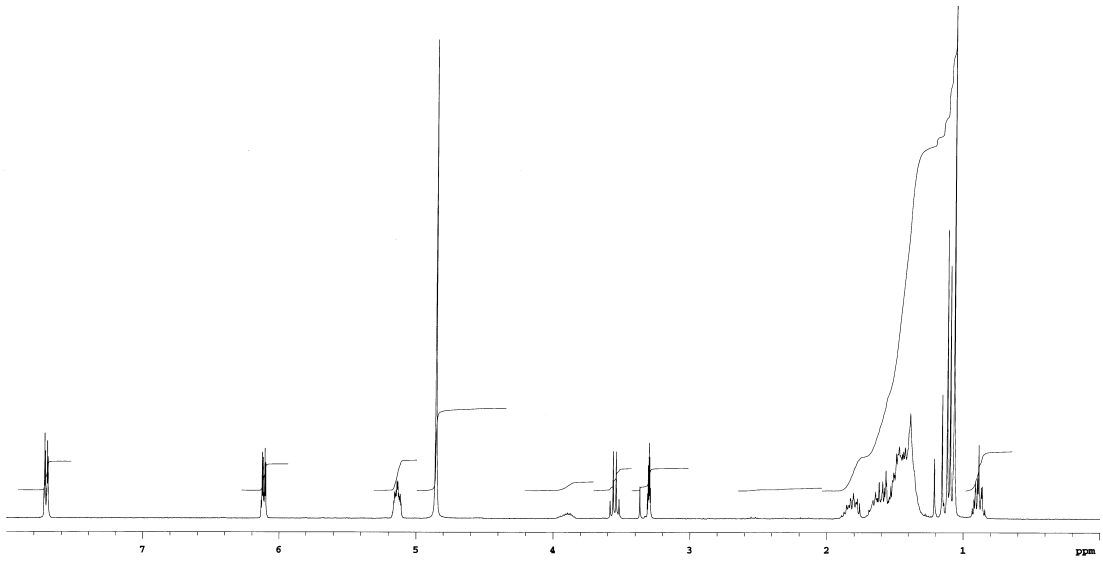


Figure 91. ^{1}H NMR spectrum (300 MHz) of 4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide (**78**) in CD_3OD.

5.6.9 4,9,10-Trihydroxy-10-methyldodec-2-en-1,4-olide

Compound **79** was isolated along with compound **78** as colourless oil, and showed the same colour reaction with anisaldehyde/sulphuric and heating. The ^{1}H NMR spectrum of compound **79** was very similar to that obtained for **78**. Preliminary examination of the ^{1}H NMR data showed that both compounds had a butenolide moiety. The ^{1}H NMR spectrum, displayed one methyl triplet at δ 0.91 connected to a methylene group in **79** instead of the methyl doublet in **78**. There was a remarkable chemical shift difference in the oxygenated region, one oxygenated methine proton appeared as quartet at 3.55 in compound 78 but one oxygenated methine proton at δ 3.30 in compound **79**. The molecular formula was established as $C_{13}H_{22}O_4$ on the basis of the HRESIMS data. The exact mass of the $[M + Na]^+$ ion matched well with the expected molecular formula of $C_{13}H_{22}O_4Na$, which was the same molecular formula as for **78**.

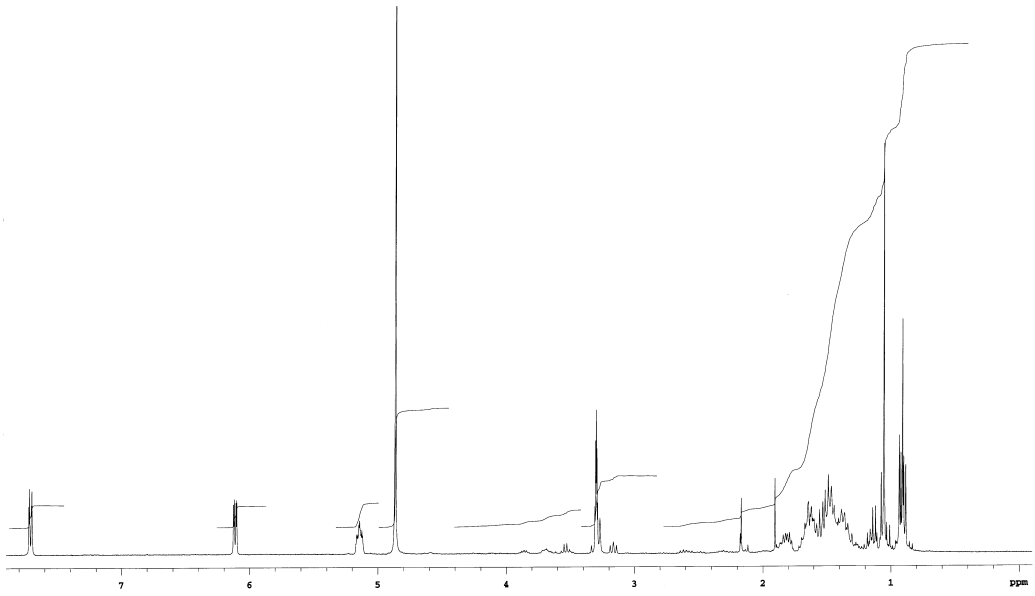


Figure 92. ^{1}H NMR spectrum (300 MHz) of 4,9,10-trihydroxy-10-methyldodec-2-en-1,4-olide (**79**) in CD_3OD.

In the ^{13}C NMR spectrum, 13 carbon signals were seen. The signal at δ 175.8 was attributed to a carbonyl of a lactone with the two sp^2 hybridized methine carbons at δ 159.6 and 121.6 forming an α,β-unsaturated carbonyl fragment. In the aliphatic region two oxygen-bearing methines at 85.5 and 78.1, oxygenated quaternary carbon signal at δ 75.5, five methylene as well as to two methyl carbon signals at δ 21.2 and 7.8 were observed. Based on the above chromatographic and spectroscopic data of compound **79** and searching in AntiBase, and the Chemical Abstracts suggested **78** as a possible structure.

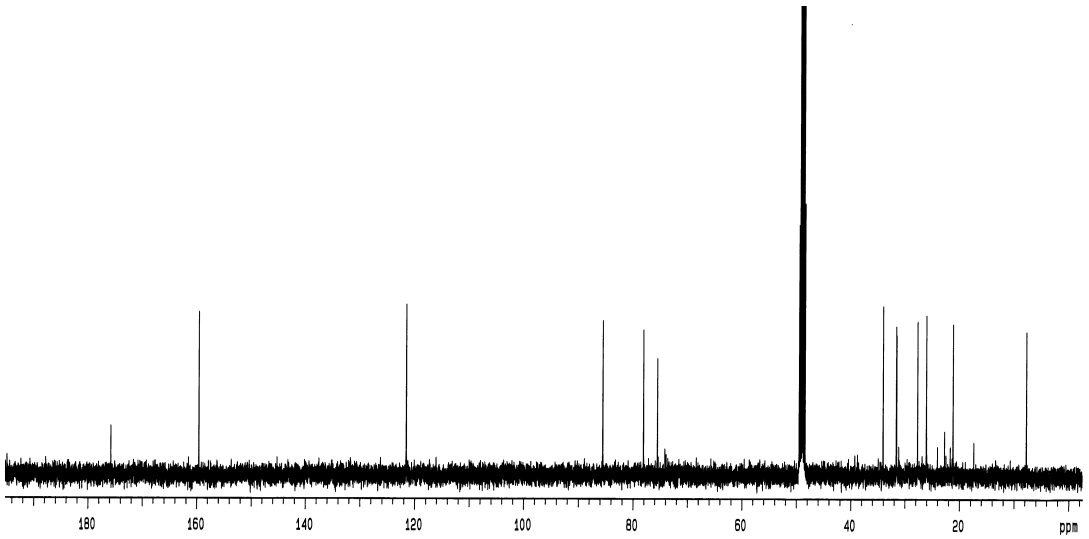
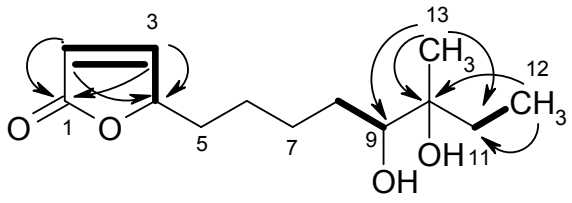


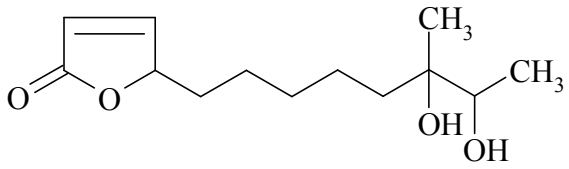
Figure 93. ^{13}C NMR spectrum of 4,9,10-trihydroxy-10-methyldodec-2-en-1,4-olide (**79**) in CD_3OD.

In the H,H-COSY spectrum the methyl triplet at δ 0.91 (H-12) showed correlation with the methylene (H-11) indicating the presence of a CH_3CH_2 fragment, so compound **78** was excluded. From the HMBC spectrum the butenolide moiety was confirmed by the correlation of the olefinic protons at δ 7.72 (H-3) and 6.12 (H-2) with the carbonyl carbon signal at δ 175.8 and the oxymethine carbon signal at 85.5. Finally both of the methyl carbon signals at δ 1.06 (H-13) and 0.91 (H-12) showed strong correlation with the methylene carbon signal at δ 31.7 (C-11) as expected. Based on the previous spectroscopic data compound **79** was established. Compound **79** was further confirmed by comparison its spectroscopic data with the related structure 5-(6,7-dihydroxy-6-methyloctyl)furan-2(5*H*)-one (**80**) which was isolated from an endophytic *Streptomyces* sp. LR4612.[139]

Butenolides are well-known metabolites in fungi,[140] bacteria[141] and higher organisms, e.g. gorgonians.[142] The saturated analogues of these compounds act as signalling substances in bacteria[143] e.g. enhancing the spore formation in streptomycetes or inducing metabolite production.[144] They are inactive in antibacterial tests.



79



80

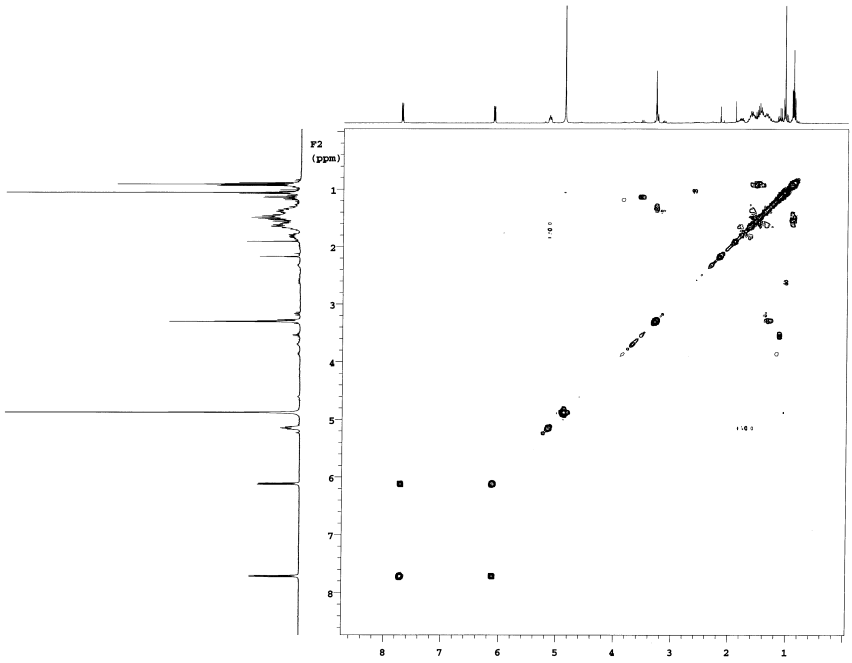


Figure 94. H,H COSY spectrum (300 MHz) of 4,9,10-trihydroxy-10-methyldodec-2-en-1,4-olide (**79**) in CD_3OD.

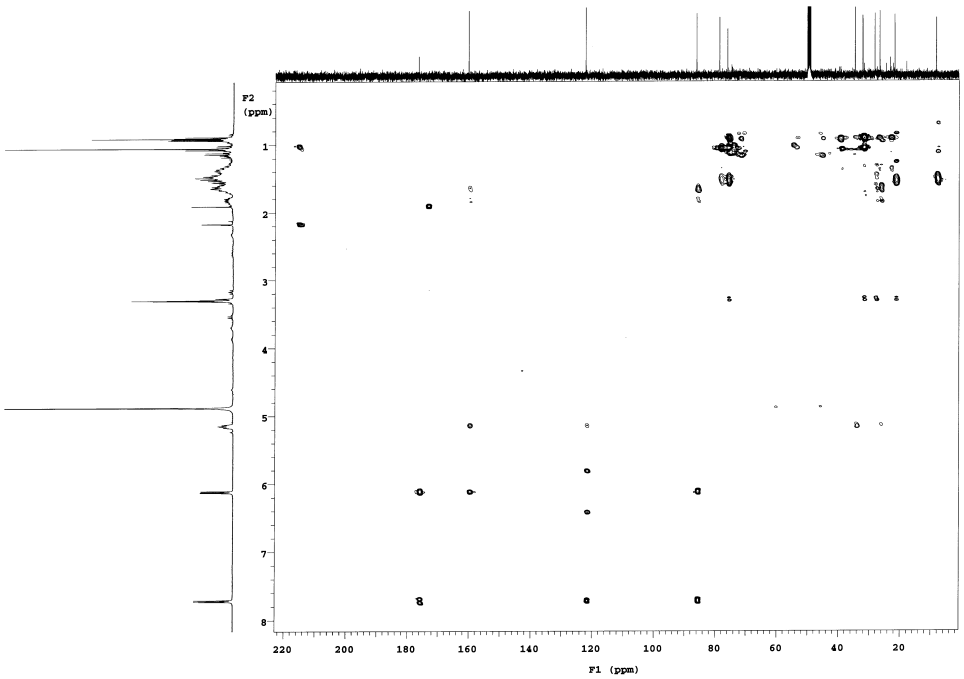
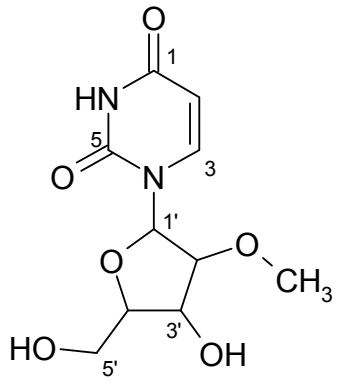


Figure 95. HMBC spectrum (300 MHz) of 4,9,10-trihydroxy-10-methyldodec-2-en-1,4-olide (**79**) in CD_3OD.

5.6.10 2´-O-Methyluridin

Purification of fraction VII (Figure 77) on Sephadex LH-20 and RP-18 delivered compound **81**. It showed strong UV absorption at 254 nm and stained to intensive greenish-blue colour with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of compound **81** displayed two 1H doublets at δ 8.08 and 5.68, assigned to an α,β-unsaturated system, as well as an anomeric 1H doublet at δ 5.93, and an up-field singlet of a methoxy group at δ 3.51. Furthermore, the spectrum exhibited three oxymethine signals at δ 4.23 (H-3'), 3.96 (H-4') and 3.84 (H-2'), as well as the ABX pattern of a methylene group at δ 3.87 and 3.73.

The ^{13}C NMR spectrum exhibited 10 carbon signals, among them two quaternary carbon signals at δ 166.2 and 152.2, which were assigned as two carbonyl groups as in the uracil system and two sp^2 carbon signals at δ 142.4 and 102.5. In addition, the spectrum contained six sp^3 carbon signals; one anomeric at 88.9, three oxygenated methines at δ 86.1, 85.1, 69.8 and one oxygenated methylene at δ 61.1, one methoxy group at δ 58.8. The (-)-ESI mass spectrum showed a *quasi*-molecular ion at *m/z* 257 ([M - H]$^-$), which fixed the molecular weight as 258 Dalton. HRESIMS delivered the molecular formula as $C_{10}H_{14}N_2O_6$. The position of methoxy group was fixed by 2D NMR experiments. The search in AntiBase with these spectroscopic data gave 2'-O-methyluridine (**81**) as the result, which was previously isolated by Schuhmann from *Streptomyces flavogriseus* ACT7651.[120]



81

The 2'-O-methylribonucleosides are present as minor components in ribonucleic acids found in rRNAs, mRNAs snRNAs and snRNAs.[145-147] The 2'- and 3'-O-methylribonucleosides exhibit resistance against enzymatic and basic hydrolysis as well as hydrolysis by phosphomonoesterases.[148] Due to these specific characteristics,

the synthesis of 2'-O-methyl derivatives are of particular interest for systems in which the cleavage of glycosidic bond affects the biological resistance of a nucleoside.[149]

5.6.11 α-Acetylamino-β-(3-indolyl)propanol

Compound **82** was easily identified as an indole derivative by the characteristic colour reaction with Ehrlich's reagent (pink). The ^{1}H NMR spectrum of compound **82** showed in the aromatic region five proton signals at δ 7.60 (ddd, H-4), 7.30 (ddd, H-7), 7.08 (m, H-6), 7.06 (s, H-2) and 6.99 (m, H-5) suggesting the presence of a 3-substituted indole such as tryptophane, indole-3-carboxylic acid etc. Furthermore, a methine signal at δ 4.19 (m, H-9), an oxymethylene at δ 3.54 (dd, 2H, CH_2-10), an ABX system of a methylene group at δ 3.01 (dd, 1H, Ha-8) and 2.89 (dd, 1H, Hb-8), as well as an acetate signal at δ 1.90 (s, H-13) were observed.

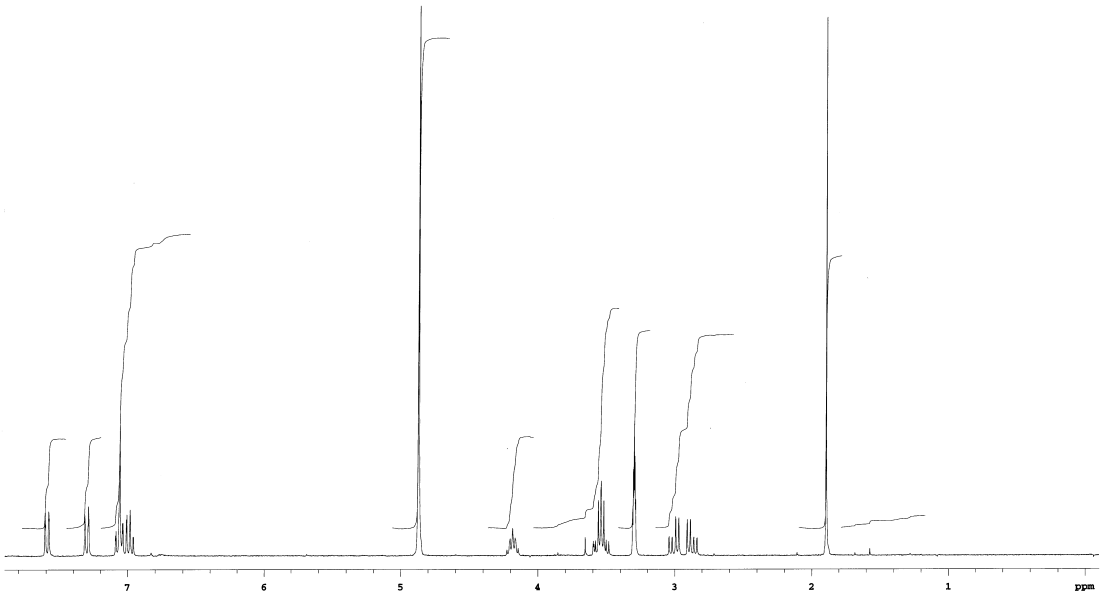
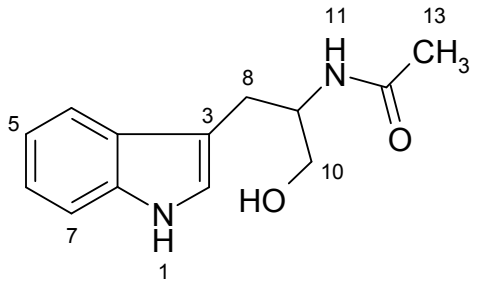


Figure 96. ^{1}H NMR spectrum of α-Acetylamino-β-(3-indolyl)propanol (**82**) in CD_3OD.

The ^{13}C NMR spectrum showed 13 carbon signals, including a carbonyl at δ 173.2, three aromatic quaternary carbons at δ 138.1, 129.1 and 112.3, five methine carbons at δ 124.0, 122.2, 119.6, 119.4 and 112.1, which confirmed the indole moiety. Finally one oxygenated carbon signal at 64.2 and an acetyl group at xxx were observed. The ESI mass spectrum confirmed the molecular weight of **82** as 232 Dalton due to the presence of two *quasi*-molecular ions at *m/z* 255 ($[M + Na]^+$) and 487 ($[2 M + Na]^+$) in the (+)-ESI mode, as well as 231 ($[M - H]^-$ in (-)-ESI mode. Compound **82** had the molecular formula $C_{13}H_{16}N_2O_2$, as determined by high-resolution ESIMS. A search in AntiBase led to α-acetylamino-β-(3-indole)propanol as a possible structure,

which was further confirmed by comparison with the literature.[150] Qing and co-workers isolated compound **82** from *Streptomyces pleomorphus* strain YIM33176 in 2005.[150]



82

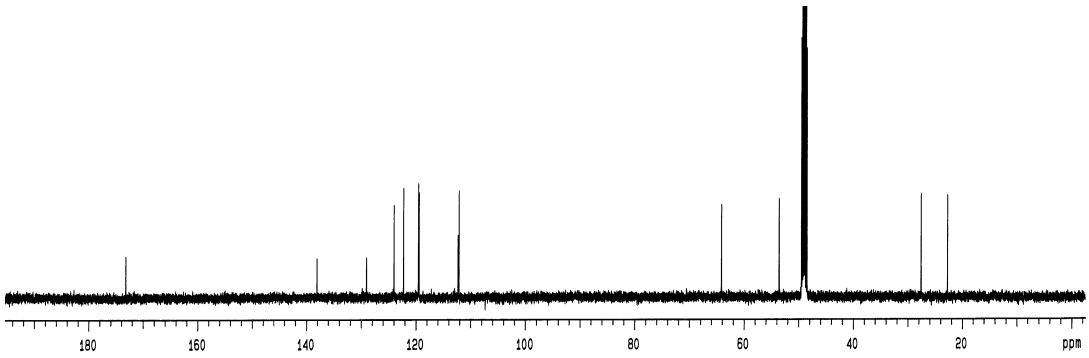


Figure 97. ^{13}C NMR spectrum of α-Acetylamino-β-(3-indole)propanol (**82**) in CD_3OD.

Table 9. Comparison of ^{1}H, ^{13}C NMR of (S)-α-acetylamino-β-(3-indole)propanol (**82**) with those reported in the literature.[150]

	Exp. $CDCl_3$		Lit. $CDCl_3$	
position	δ_H	δ_C	δ_H	δ_C
2	7.06	124.0	7.06	124.2
3	-	112.3	-	112.3
3a	-	129.1	-	129.1
4	7.60	119.4	7.60	119.6
5	6.99	119.6	7.00	119.8
6	7.08	122.2	7.08	122.3
7	7.30	112.1	7.30	112.2
7a	-	138.1	-	138.0
8	2.89 3.01	27.6	2.89 3.01	27.6
9	4.19	53.6	4.20	53.6
10	3.54	64.2	3.52	64.2
12	-	173.2	-	173.2
13	1.90	22.8	1.89	22.8

5.6.12 Pyridine-3-carboxylic acid; Nicotinic acid

Compound **83** was obtained as a colourless solid from fraction VII by subjecting it to Sephadex LH-20 followed by RP18. It showed a middle polar UV absorbing zone, which turned to faint pink with Ehrlich's reagent. The ^{1}H NMR spectrum displayed in the down field shift a singlet at δ 9.02 (H-2), as well as to three other protons with *o*-couplings were observed at δ 8.68 (d, ^{3}J = 4.1 Hz), 8.28 (ddd, ^{3}J = 8.0, ^{4}J = 1.7, ^{4}J = 1.8 Hz) and 7.54 (dd, ^{3}J = 5.0, ^{3}J = 4.9 Hz). Due to the observed high proton shifts and the small coupling constants, the compound must consist of a hetero-aromatic ring.

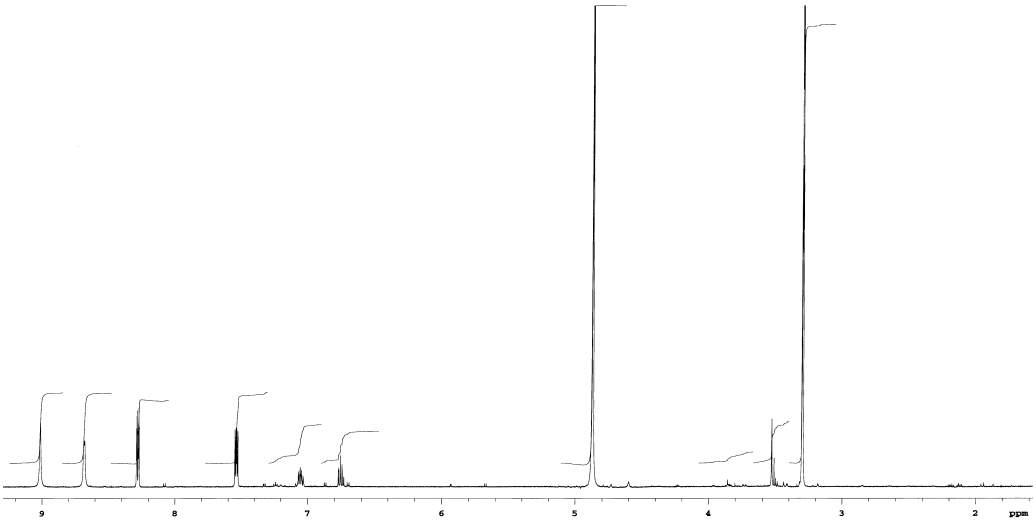
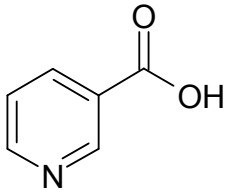


Figure 98. ^{1}H NMR spectrum (300 MHz) of nicotinic acid (**83**) in CD_3OD.

In the ^{13}C NMR spectrum six carbon signals were observed in sp^2 region. One carbonyl at δ 169.8, two at δ 152.8 and 149.5, which could be connected to a nitrogen atom, and another two carbons at δ 137.3, 125.1 were present. The H,H COSY spectrum showed a strong correlation between the proton at δ 7.54 (H-5) with those at δ 8.68 (H-2) and 8.28 (H-6) which both correlated to that at δ 9.02. The careful interpretation of the HMBC data led to the structure of nicotinic acid.



83

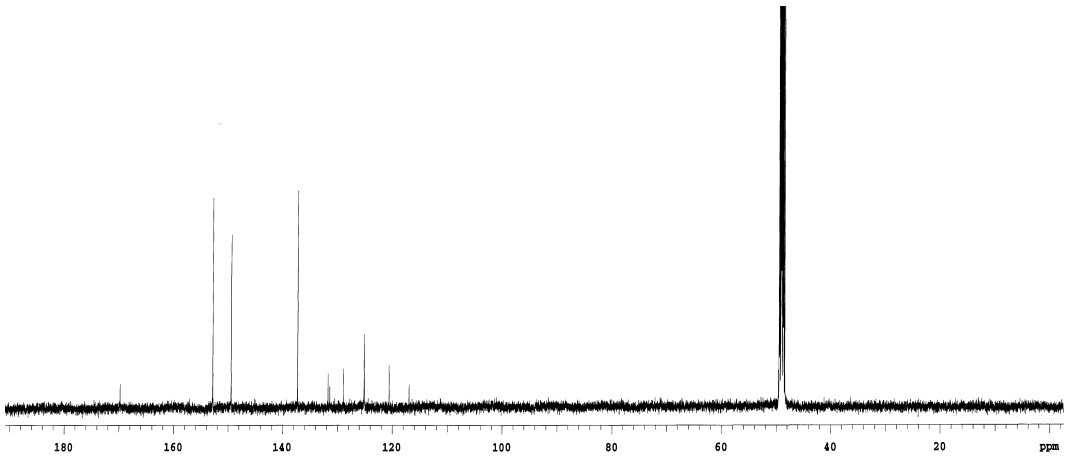


Figure 99. ^{13}C NMR spectrum (125 MHz) of nicotinic acid (**83**) in CD_3OD.

Nicotinic acid (**83**), which is also known as niacin (**83**), was reported as a fungal metabolite from *Phycomyces blakesleeanu*[151] and is an important plant intermediate. It is an enzyme cofactor, vasodilator and antihyperglycemic agent and widely used in treatment of lipid disorders.[152]

6 Terrestrial *Streptomyces* spp.

6.1 *Streptomyces* sp. GT 2005/ 014

The antimicrobial assay of the crude extract *Streptomyces* sp. GT 2005/ 014 showed moderate activity against *Streptomyces viridochromogenes* (Tü 57) and *Mucor miehei* (Tü 284) but no activity against other bacteria tested. It showed 92% activity against *Artemia salina.* TLC showed various non-UV active zones by a strong violet colour with anisaldehyde/sulphuric acid. In addition, HPLC/MS showed the presence of nonactic acid and homononactic acid, bafilomycin B_1 (**85a**) and B_2 (**85b**).

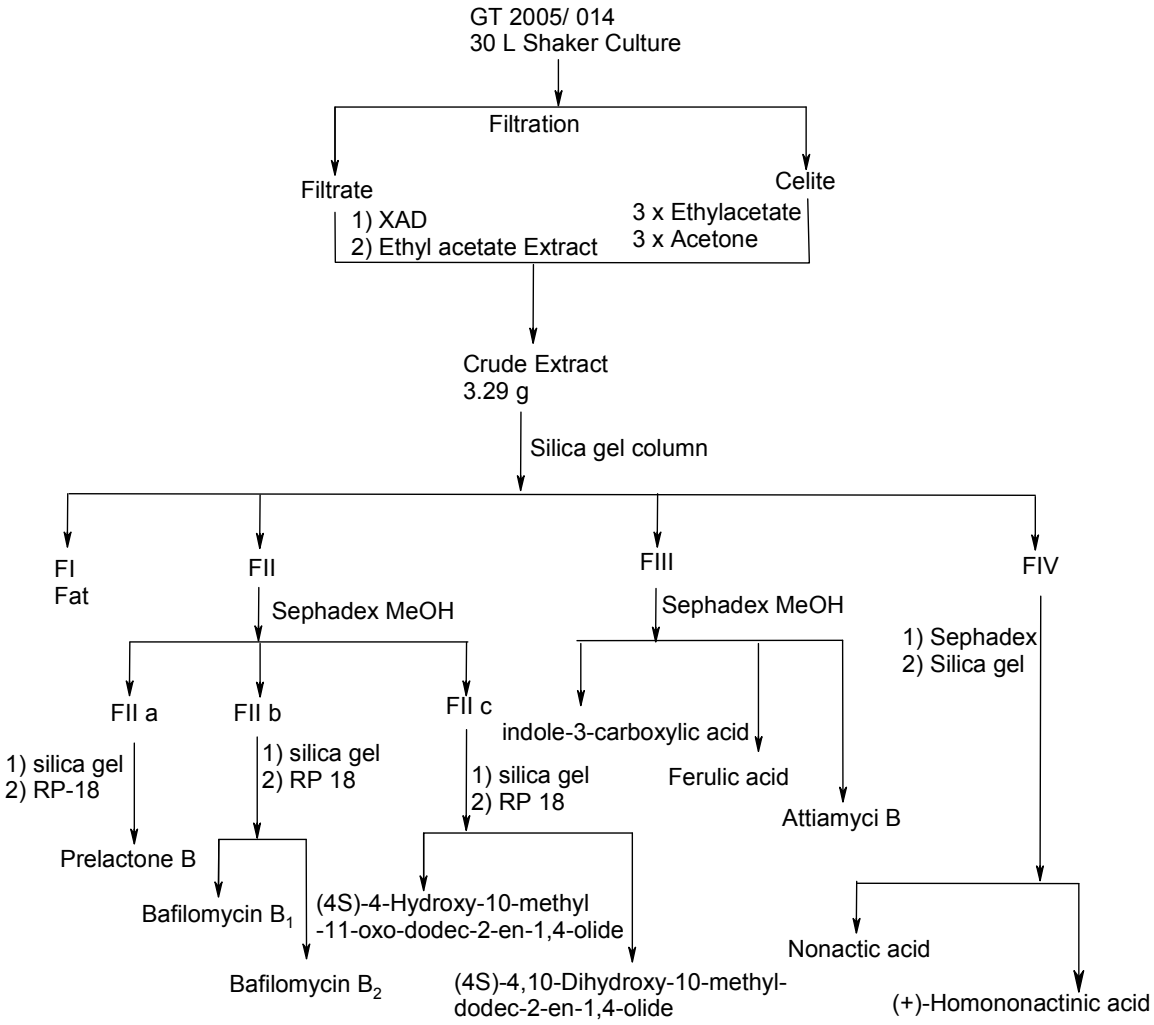


Figure 100. Work-up scheme of *Streptomyces* sp.GT 2005/ 014.

6.1.1 Prelactone B

Subjecting the sub fraction II (Figure 100) to Sephadex LH-20 and then RP-18 afforded prelactone B (**84**). The ^{1}H NMR spectrum of this compound showed three methyl doublets at δ 0.93, 1.08 and 1.10. There were four methine proton signals, two oxygenated at δ 3.77 and the other two at δ 2.01 and 1.76. Finally the spectrum showed one ABX system at δ 2.92 and 2.49, which seemed to be near a chiral centre. ESIMS revealed the molecular ion peak at *m/z* 172, and HRESIMS gave the molecular formula $C_9H_{16}O_3$.

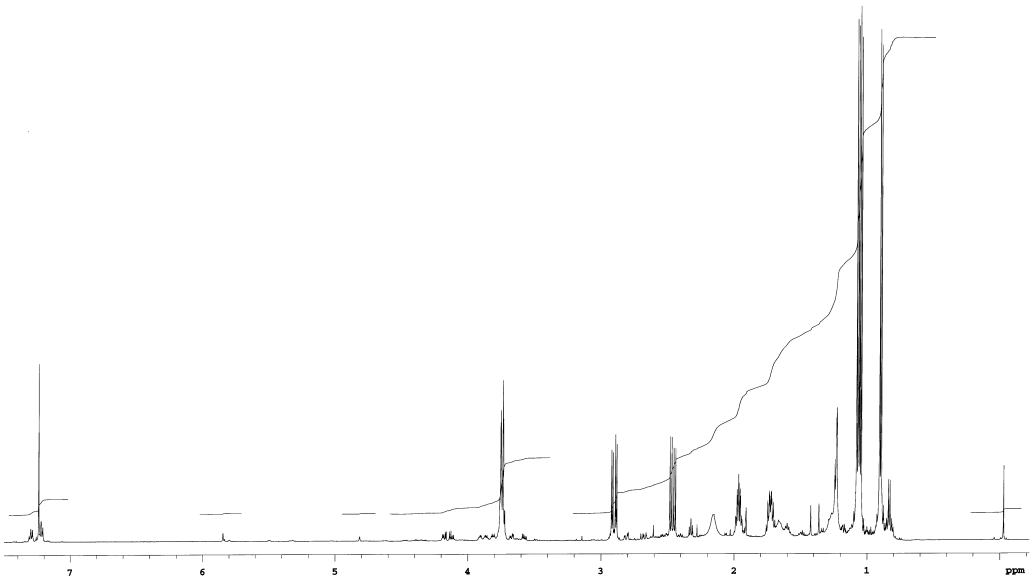


Figure 101. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of prelactone B (**84**).

From the ^{13}C NMR and HSQC spectra, there were in total 9 carbon signals, one carbonyl of acid, ester or amide at δ 171.1 (CO-2), two oxygenated carbon signals at δ 86.3 (C-6) and 69.7 (C-4), respectively, and six sp^3 carbon signals (one methylene, two methine and three methyl carbons). From the HSQC spectrum, it was clear that the protons at δ 2.92 and 2.49 were connected to the same carbon at δ 38.9 corresponding to an ABX system. Searching in AntiBase with the obtained spectroscopic data resulted in prelactone B (**84**).

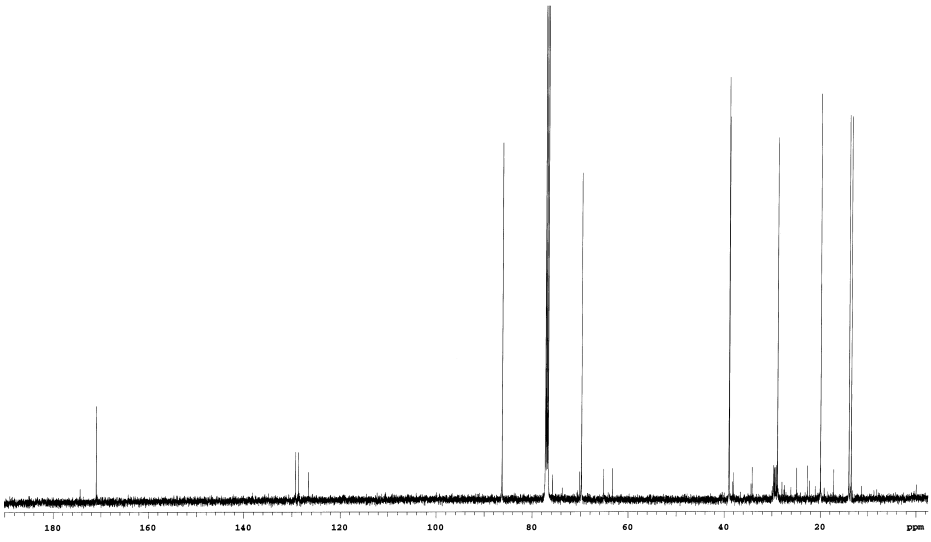


Figure 102. ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of prelactone B (**84**).

To confirm the structure of compound **84**, 2D NMR experiments were performed. In the H,H COSY spectrum, one methine proton at δ 2.01 showed ^{3}J correlations with two methyls at δ 0.93 and 1.10 to construct an isopropyl moiety ($(CH_3)_2CH$) and with the oxygenated methine proton at δ 3.77. The third methyl group showed ^{3}J correlation with one methine proton at δ 1.76 and the latter again with the oxygenated methine proton at δ 3.77, thus resulting in prelactone B (**84**)

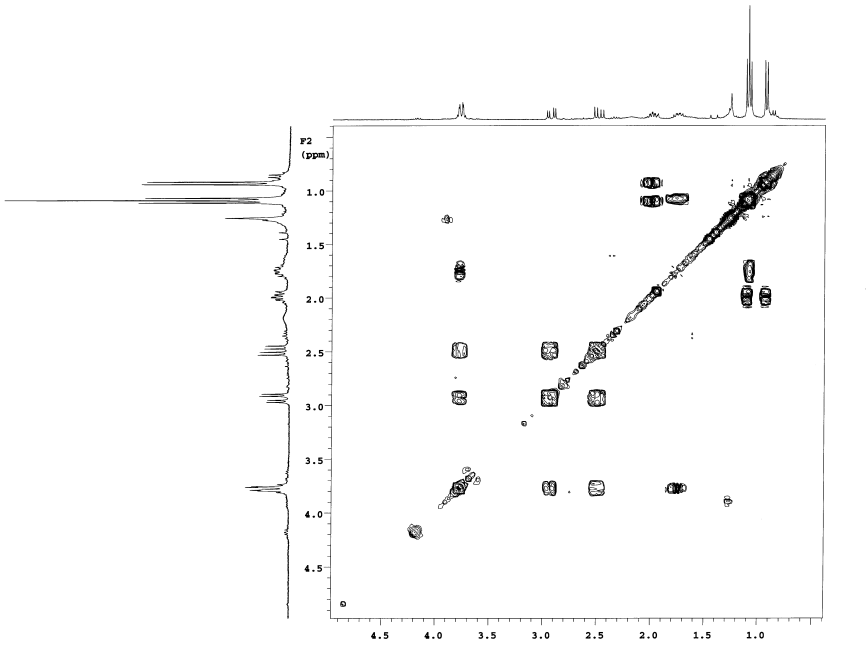


Figure 103. H,H COSY spectrum ($CDCl_3$, 300 MHz) of prelactone B (**84**).

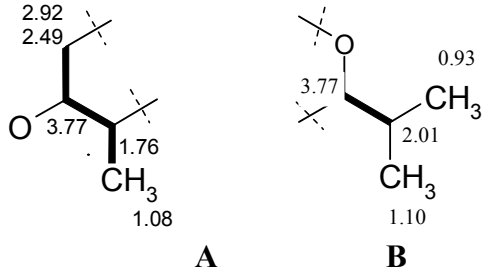


Figure 104. H,H COSY correlations of the fragments of prelactone B (**84**).

From HMBC spectrum, the two methyl doublets at δ 1.10 and 0.93 showed strong correlations with the methine carbon at 28.9 (C-1') and the oxygenated methine carbon at δ 86.3 (C-6). Furthermore, the oxygenated methine at 3.77 (H-6) and the methylene at 2.49 and 2.92 (ABX) showed correlations with the carbonyl at δ 171.1 and the methine at 1.76 which connected fragment A and B via CO-2 and C-5 and resulted in prelactone B (**84**) .

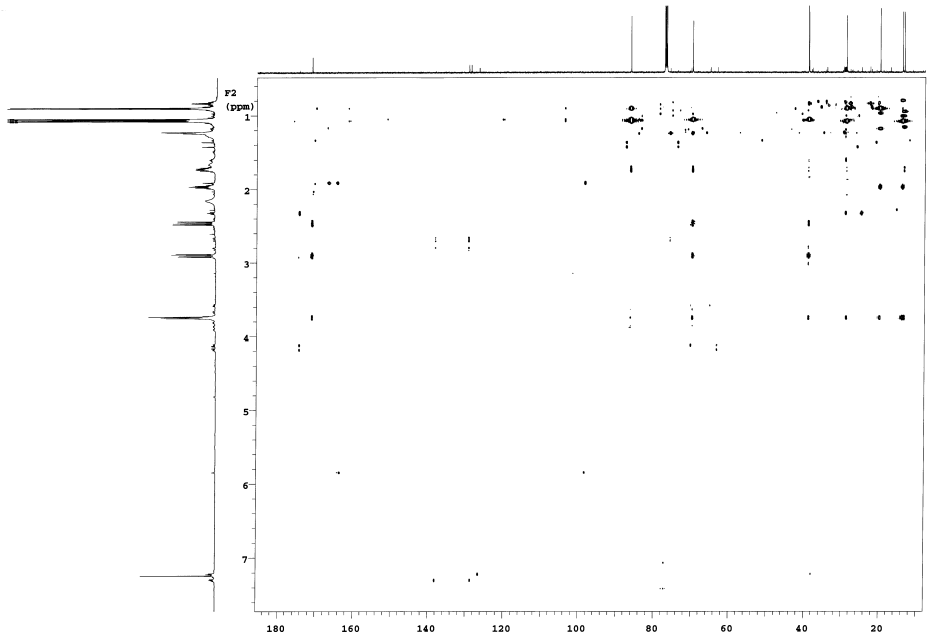
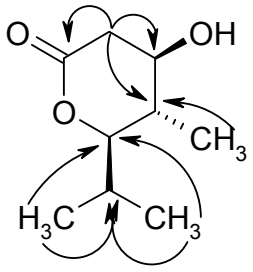


Figure 105. HMBC spectrum ($CDCl_3$, 300 MHz) of prelactone B (**84**).

The structure was further confirmed by comparison the above spectroscopic data with the published one. Prelactone B (**84**) was isolated from the concanamycin-producing *Streptomyces sp* (strain Go 22/15) and bafilomycin-producing *Streptomyces griseus* (strain Tü 2599).[153] Prelactone B (**84**) is identical in ring size, substituent pattern, and configuration, having the 6-membered hemiacetal portion of the bafilomycins B_1(**85a**) and B_2 (**85b**).



84

6.1.2 Bafilomycins

The sub-fraction FII-b showed several UV absorbing bands at 245 nm, which, turned to reddish-brown by anisaldehyde/sulphuric acid after heating. This fraction was subjected to silica gel column followed by PTLC to afford two yellow solid components; bafilomycin B_1 (**85a**) and B_2 (**85b**)

6.1.2.1 Bafilomycin B_1

The ^{1}H NMR spectrum of **85a** showed two H/D exchangeable protons at δ 13.51 and 9.18. There was a large number of proton signals in the olefinic and oxygenated methine region. Firstly, the spectrum displayed two doublets at δ 7.25 and 6.89 with the coupling constant of 15.1 Hz for protons at a *trans* double bond. In the ^{1}H NMR spectrum, five olefinic methine protons were observed at δ 6.70 (s), 6.57 (dd), 5.82-5.80 (t, 2H), 5.18 (dd). In the aliphatic region, there were five oxygenated methine protons at δ 4.98, 4.18, 3.91, 3.61 and 3.32. Additionally, two methoxy groups at δ 3.62 and 3.22 and two methyl singlets at δ 1.99 and 1.95 were observed, which are possibly connected to sp^2 carbons. Multiplets of four methylene groups between δ 1.20-2.58 and seven methyl doublets between d 0.78-1.20 appeared.

ESIMS revealed the molecular ion peak at *m/z* 815. A search in AntiBase by using above spectroscopic data as well as the molecular weight, the isolated compound was identified as bafilomycin B_1 (**85a**). It was further confirmed by comparing the spectrum with an authentic spectrum as well as with literature data.

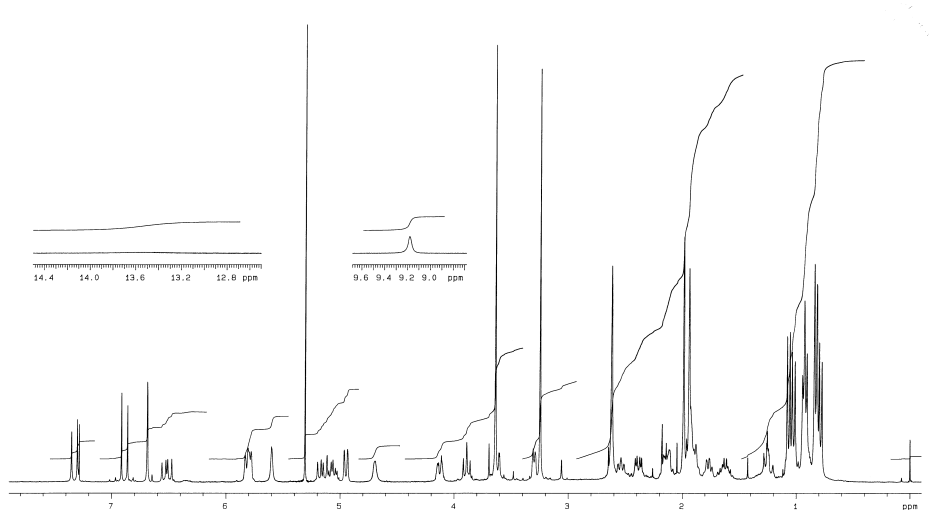
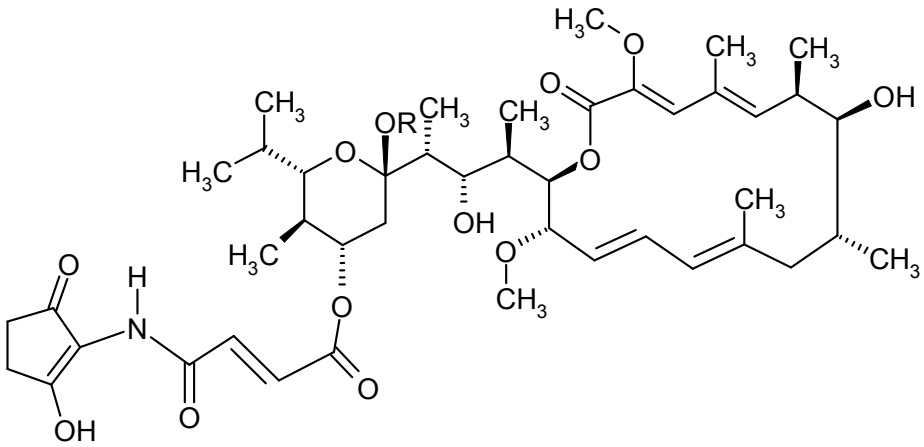


Figure 106. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of bafilomycin B_1 (**85a**).

6.1.2.2 Bafilomycin B_2

The ^{1}H NMR spectrum of bafilomycin B_2 (**85b**) was similar to that of bafilomycin B_1 (**85a**). However the spectrum of bafilomycin B_2 (**85b**) showed an additional methoxy group at δ 3.06. ESIMS of this component gave the molecular ion peak at 829 Daltons. Bafilomycin B_1 (**85a**) is a natural product whereas bafilomycin B_2 (**85b**) is formed during the isolation procedure.[154]



85a R = H **85b** R = Me

Antibiotics of the bafilomycin family are known since several years to possess a broad spectrum of biological activity against Gram-positive bacteria, fungi, yeasts, insects, protozoa, cestodes, and free-living nematodes.[155] More recently, their potent nematocidal activity against the free-living stages of intestinal parasitic nematodes has received importance in the animal health industry.[156] Furthermore the bafilomycins have been suggested as possible therapeutic agents in the treatment of peptic ulcera, by virtue of their inhabitation of cell vacuolisation induced by the bacterium *Helicobacter pylori*.[157-159] The molecular structure of the bafilomycins are characterised by the presence of a 16-membered macrolide nucleus to which various side chains are attached at the lactone terminus (C15), leading to the generic term bafiloid for these antibiotics.[160] The macrolides bafilomycin A_1, B_1, are potent and specific inhibitors of V-ATPases.[161] Bafilomycin B_1 (**85a**) shares the same mode of action and activity spectrum as its more readily accessible A_1 analogue. It has also attracted interest as a potential antiosteoporotic agent for the treatment of bone lytic diseases.[162]

Bafilomycins A_1, A_2, B_1, B_2, C_1, C_2, were first isolated in 1983 by Werner and Hagenmaier[163] from the culture of *Streptomyces griseus* ssp. *sulphurus* as a new type of antibiotics that exhibited activity against Gram-positive bacteria and fungi, and due to its high activity, a total synthesis was developed by K. Toshima.[164]

6.1.3 4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide

Sub fraction II-C showed no UV absorbing bands in the nonpolar region but turned to violet and red with anisaldehyde/sulphuric acid and heating. These two compounds were isolated as colourless oil from sub fraction IIc by eluting it from a silica gel column and then followed by RP-18 column separation. In ^{1}H NMR spectrum of **86** there were three signals at δ 7.44 (dd), 6.12 (dd) and 5.04 (m), which are

typical for butenolide moiety. In the aliphatic region, one methyl triplet at δ 0.90, one methyl singlet at 1.12 and the multiplet of six methylene groups between 1.43 and 1.70 were also observed.

ESIMS showed a molecular ion peak at *m/z* 226. A search in AntiBase by using above spectroscopic data as well as molecular weight, the isolated compound was assigned as 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**86**).

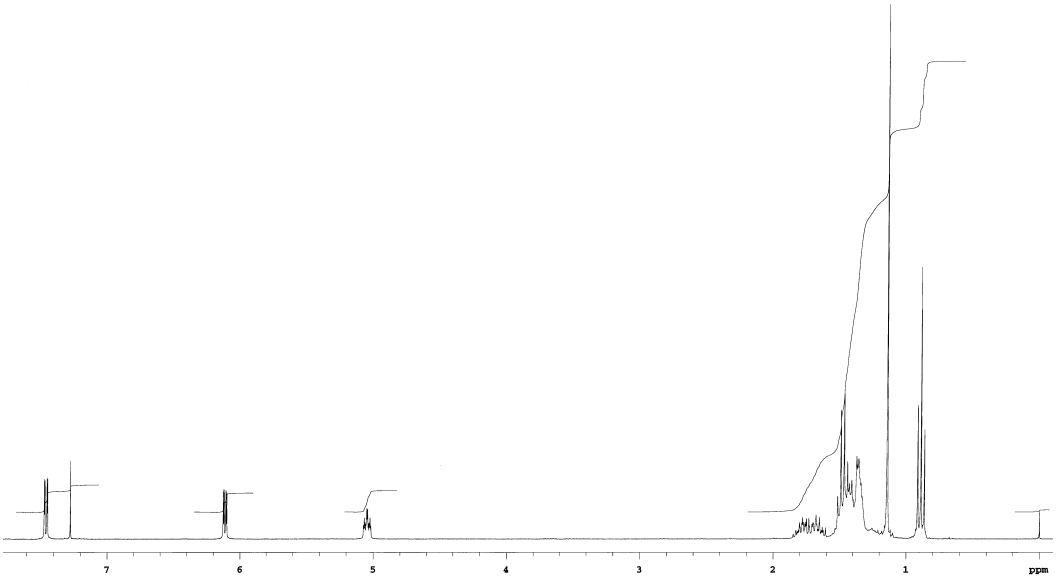
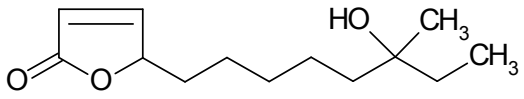


Figure 107. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**86**).

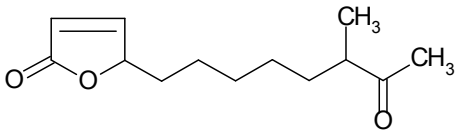


86

6.1.4 4-Hydroxy-10-methyl-11-oxododec-2-en-1,4-olide

Compound **87** showed non UV absorbing zone and gave a red colour reaction with anisaldehyde/sulphuric acid after heating. The ^{1}H NMR spectrum of this compound exhibited again the butenolide moiety. In the aliphatic region, the spectrum revealed a methyl doublet at δ 1.05, the methyl singlet of an acetyl group at δ 2.05, overlapping multiplet signals between δ 1.2 and 2.0 for five methylene groups and a methine multiplet at δ 2.45 which is possibly attached to a sp^2 carbon. ESIMS revealed the molecular ion peak at *m/z* 224. The difference in the molecular weight of $\Delta m = 2$ between **86** and **87** suggested that **87** has one double bond more. By a search in AntiBase using the above spectroscopic data, the isolated compound was assigned as 4-hydroxy-10-

methyl-11-oxo-dodec-2-en1,4-olide. The structures of compound **86** and **87** were further confirmed by comparison with the literature[165] data, and authentic spectra.



87

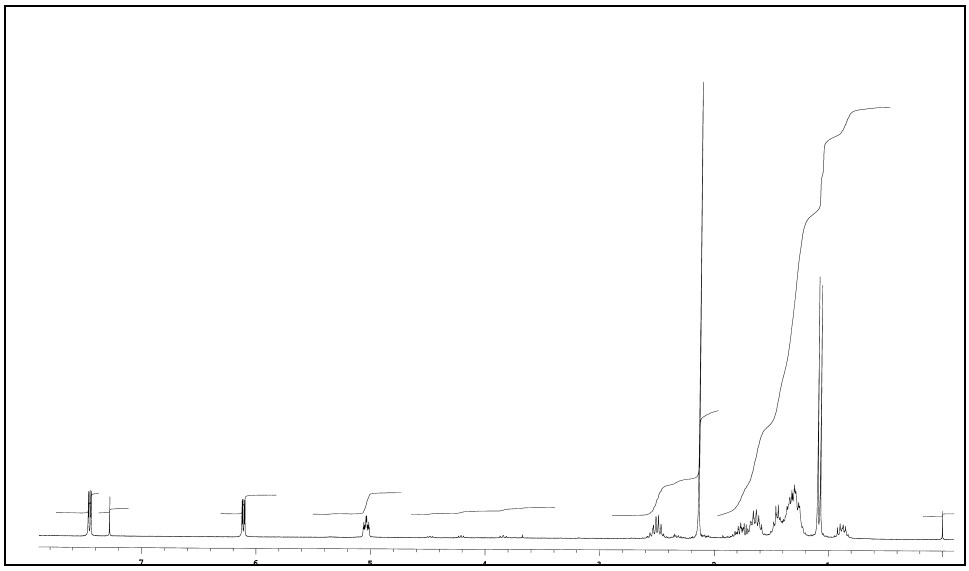
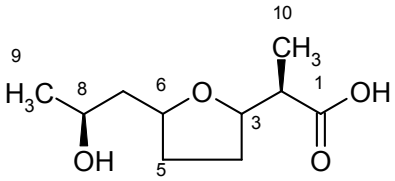


Figure 108. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**87**).

6.1.5 Nonactic acid

Compound **88** was isolated as colourless oil from fraction IV by subjecting it to Sephadex LH-20 followed by silica gel. Fraction IV was showed a colourless spot on TLC, which turned to violet on spraying with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum indicated signals for 18 protons only in the aliphatic region of the spectrum. It contained two doublets of methyl groups at δ 1.17 (H-10) and δ 1.21 (H-9), multiplets at δ 1.68 (4 H), 2.02 (2H), one methine quartet at δ 2.50 and three oxygenated methine signals at δ 3.99 (H-3) 4.08 (H-8) and 4.22 (H-6) respectively. ESIMS of this compound afforded a molecular ion peak at *m*/*z* 202, and HRESIMS revealed the molecular formula $C_{10}H_{18}O_4$. Searching in AntiBase using the molecular weight, the molecular formula and the ^{1}H NMR data led to (±)-nonactic acid (**88**).



88

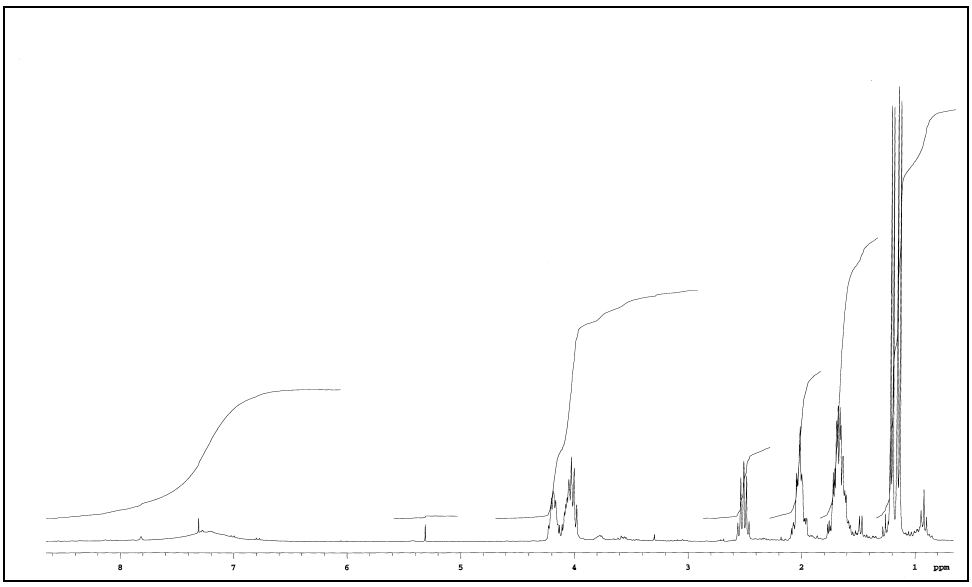


Figure 109. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of nonactic acid (**88**).

Nonactic acid has been synthesised in 2006 by P. A. Bercedo.[166] It exhibited moderate 3α-hydroxysteroiddehydrogenase inhibiting activity.[167]

6.1.6 Ferulic acid

When fraction III was subjected to Sephadex LH-20 with methanol, ferulic acid (**89**) was isolated. It showed UV absorbing band at 256 nm and turned to violet with anisaldehyde/sulphuric acid and heating.

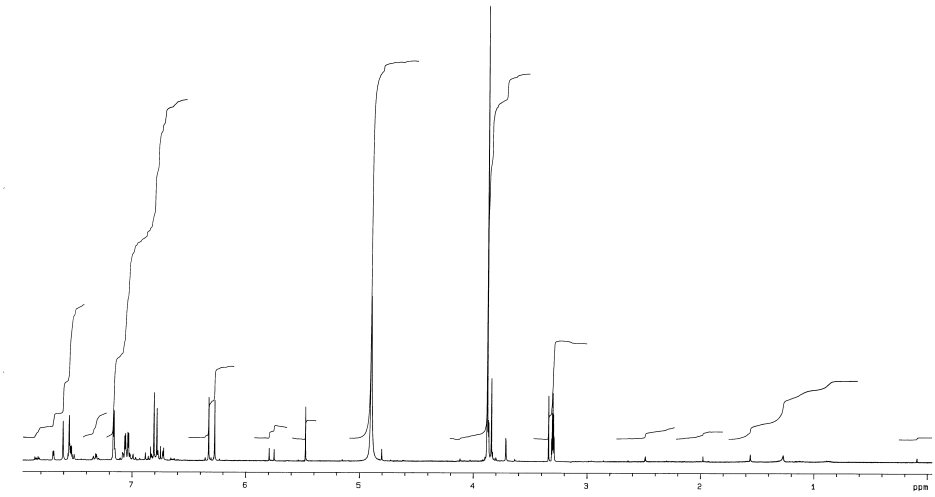
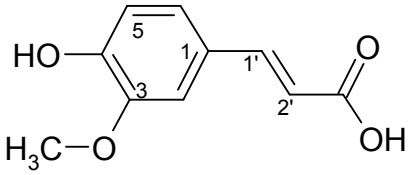


Figure 110. ^{1}H NMR spectrum (CD_3OD, 300 MHz) of ferulic acid (**89**).

^{1}H NMR spectrum showed two doublets at δ 7.58 and δ 6.30 for the *trans*-disubstituted α,β-unsaturated carbonyl. In the aromatic region, the proton at δ 7.05 showed an *ortho*-coupling with the proton at δ 6.80 and a *m*-coupling with the proton at δ 7.16. From these spectroscopic data, the compound contained an ABX system or a 1,3,4-trisubstituted aromatic ring. In the aliphatic region, the spectrum revealed the methoxy group at δ 3.88. ESI MS revealed the molecular ion peak at *m/z* 193.0 for the $[M - H]^-$ peak. A search in AntiBase using the above spectroscopic data as well as molecular weight assigned the isolated compound was as ferulic acid (**89**), which has been further confirmed by comparing with authentic spectra.



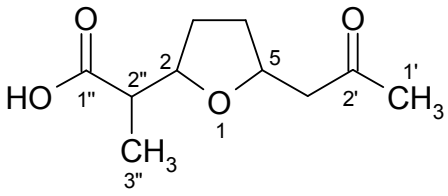
89

6.1.7 Attiamycin B

Attiamycin B (**90**) was obtained from fraction III on Sephadex LH-20 as colourless, non UV-absorbing oil, which gave a violet colour with anisaldehyde/sulphuric acid. The molecular weight of attiamycin was deduced from ESIMS as *m/z* 200, corresponding to $C_{10}H_{16}NO_4$, with three double bond equivalents which is one more than the structurally related nonactic acid.

The ^{1}H NMR spectrum revealed no aromatic or olefinic protons, which indicated a merely aliphatic compound. The spectrum exhibited an exchangeable OH proton at

5.85, two multiplets of oxy-methine protons were observed at δ 4.28 and 4.06. In the high field region between 2.80~1.14, several multiplets were observed with integration of 7 protons, divided into two categories, three methylene groups and one methine proton at δ 2.52, in the same region two methyl groups were observed one as singlet at δ 2.18, which may be connected to an sp^2 carbon or sulphur, the other methyl was doublet at δ 1.15. Finally, searching in AntiBase using the ^{1}H NMR and the MS data resulted in attiamycin B (**90**)



90

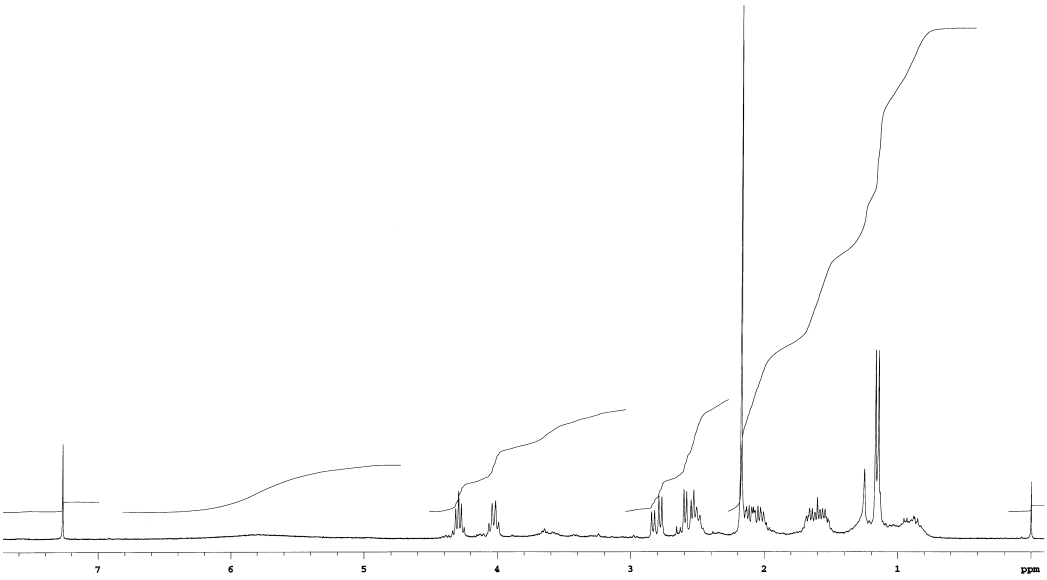


Figure 111. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Attiamycin B (**90**).

Attiamycin B, a new marine secondary metabolite has been in parallel isolated from the marine Actinomycete isolate Act8970 and *Streptomyces* sp. B8289, and it showed a moderate activity against *Staphylococcus aureus*.[168] Attiamycin B (**90**) is the oxidised form of nonactic acid with the hydroxyl group replaced by a carbonyl.

6.2 Terrestrial *Streptomyces* sp. Gt-2005/009

The pre-screening of the crude extract of the terrestrial *Streptomyces* sp. Gt-2005/009 showed a high biological activity against *Streptomyces viridochromogenes* (Tü57), *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Chlorella vulgaris*,

Chlorella sorokiniana, *Candida albicans* and *Mucor miehei*. In addition, the chromatogram showed non-polar and non UV absorbing bands which showed a violet colour on spraying with anisaldehyde/sulphuric acid. Furthermore, yellow-orange spots appeared, which showed no colour change with NaOH but changed to red with anisaldehyde/sulphuric acid and suggested the presence of actinomycins. These were identified by HPLC/MS as actinomycin X_2 (*m/z* 1269) and D (*m/z* 1255).

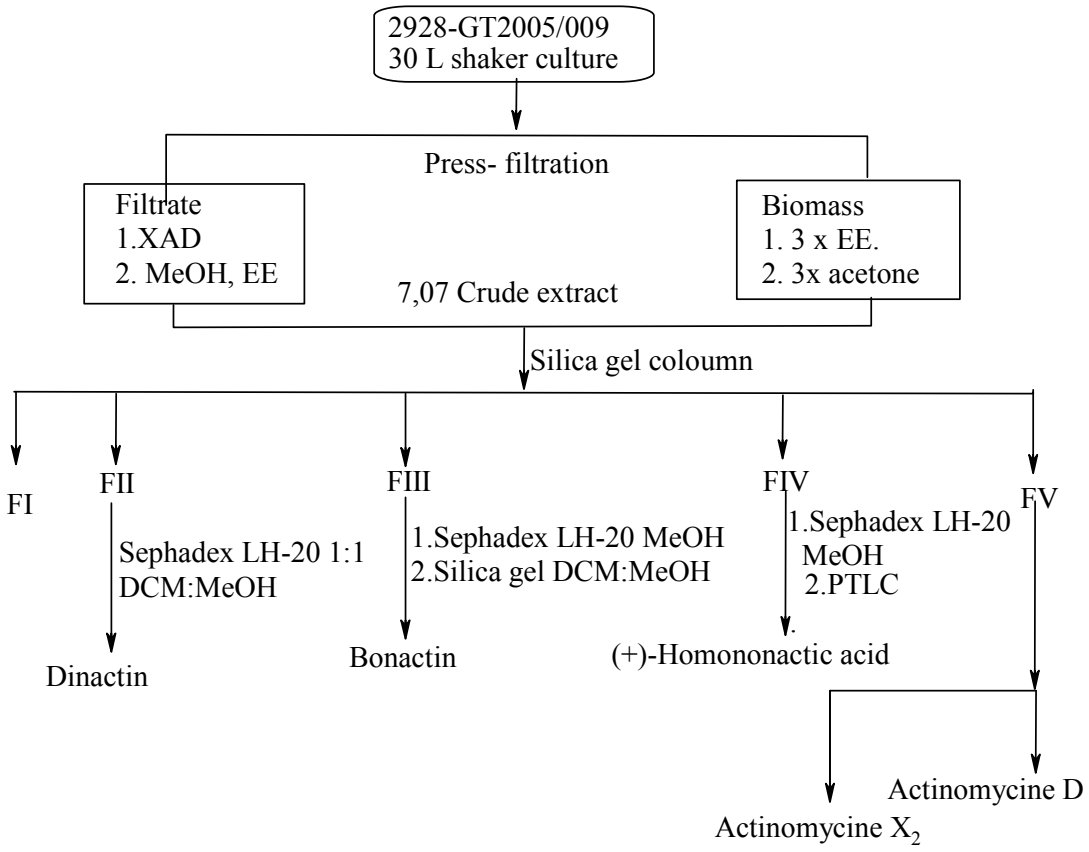


Figure 112. Work up scheme of the terrestrial *Streptomyces* sp. Gt-2005/009.

6.2.1 Dinactin

Further separation was carried out on fraction II to afforded colourless oil, which appeared as non UV absorbing band and showed a violet colour with anisaldehyde/sulphuric acid. The compound showed the same physical and chemical properties as nonactic acid and homononactic acid so it was expected to have similarities in its structure with them.

The aliphatic region of the ^{1}H NMR spectrum showed three proton signals at δ 4.93 (H-8,17), 4.02 (H-3,12) and 3.87 (H-6,15), which were interpreted as oxygenated methines. Furthermore, one multiplet at δ 2.52 with the intensity of 2H possibly con-

nected to sp^2 carbon and seven methylene multiplets in the range of δ 2.01-1.40 were displayed.

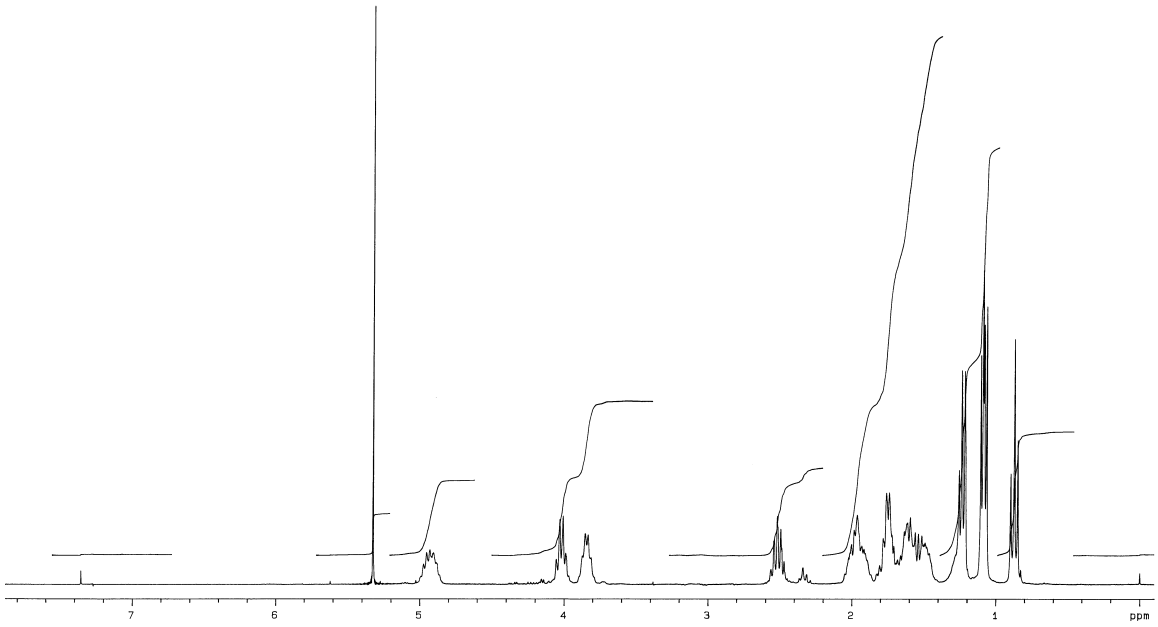


Figure 113. ^{1}H NMR spectrum (300 MHz) of dinactin (**91**) in $CDCl_3$.

The ^{13}C NMR spectrum exhibited signals of 21 carbon atoms, which could be classified according to the HSQC spectrum as follows: two carbonyls at δ 174.2, 173.9, six oxygenated methine groups in the range of δ 79.8-68.8, two methine groups connected to sp^2 carbon at δ 45.0 and 44.8. Seven methylenes groups at δ 42.0- 27.1, and four methyls, at δ 20.2, 2 × 12.9, 9.1 were present.

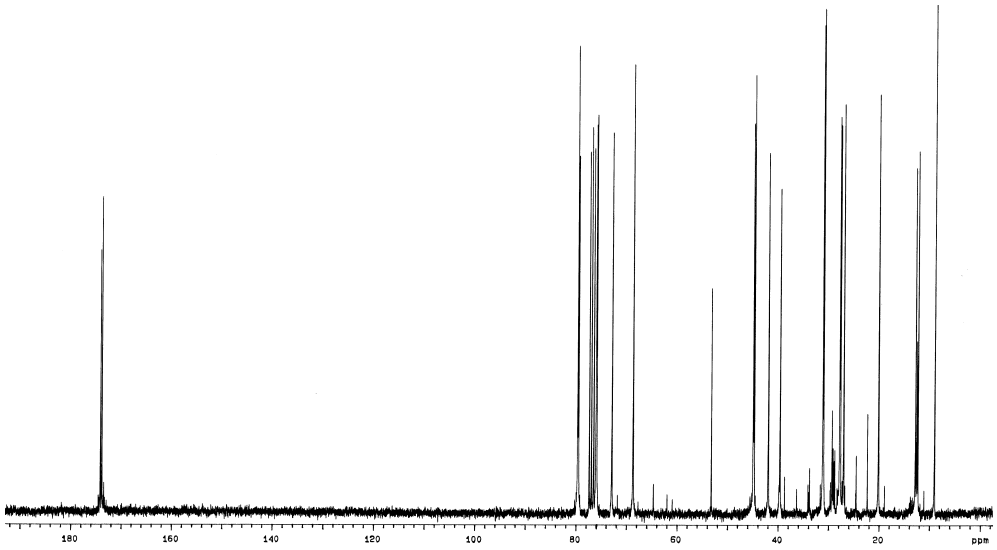


Figure 114. ^{13}C NMR spectrum (75 MHz) of dinactin (**91**) in $CDCl_3$.

Table 10. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data of dinactin (**91**).

Position	Dinactin				
	^{1}H (Int., mult, *J* [Hz])	^{13}C		^{1}H (Int., mult., *J*Hz])	^{13}C
1	-	174.2	11	2.52 (2H, m)	45.0
2	2.52 (1H, m)	44.8	CH_3-11	1.10 (3H, d, 7.0)	-
CH_3-2	1.08 (3H, d, 7.0)	12.9	12	4.02 (2H,m)	79.8
3	4.02 (1H, m)	79.6	13a	1.92 (2H, m)	27.9
4a	1.92 (1H, m)	27.9	13b	1.56 (2H, m)	-
4b	1.56 (1H, m)	-	14a	2.01 (2H, m)	31.1
5a	2.01 (1H, m)	31.2	14b	1.49 (2H, m)	-
5b	1.49 (1H, m)	-	15	3.87 (1H,m)	76.0
6	3.87 (1H,m)	76.1	16		39.7
7a	1.67 (2H m)	42.0	17	4.93 (2H, m)	72.9
8	4.93 (2H, m)	68.8	18	1.56 (2H, m)	27.1
CH_3-8	1.23 (3H, d, 6.2)	20.2	19	0.87 (3H, t,7.4)	9.1
10	-	173.9			

Compound **91** revealed an $[M + Na]^+$ peak at 787.0 in the (+)-ESI spectrum, suggesting the molecular formula of $C_{42}H_{68}O_{12}$. The ^{1}H NMR and ^{13}C NMR spectra showed half of the number of protons and carbons as expected from the formula, which indicated two symmetrical parts. Searching in AntiBase using the above spectroscopic data led to dinactin (**91**). The structure **91** was finally confirmed by 2D NMR measurements.

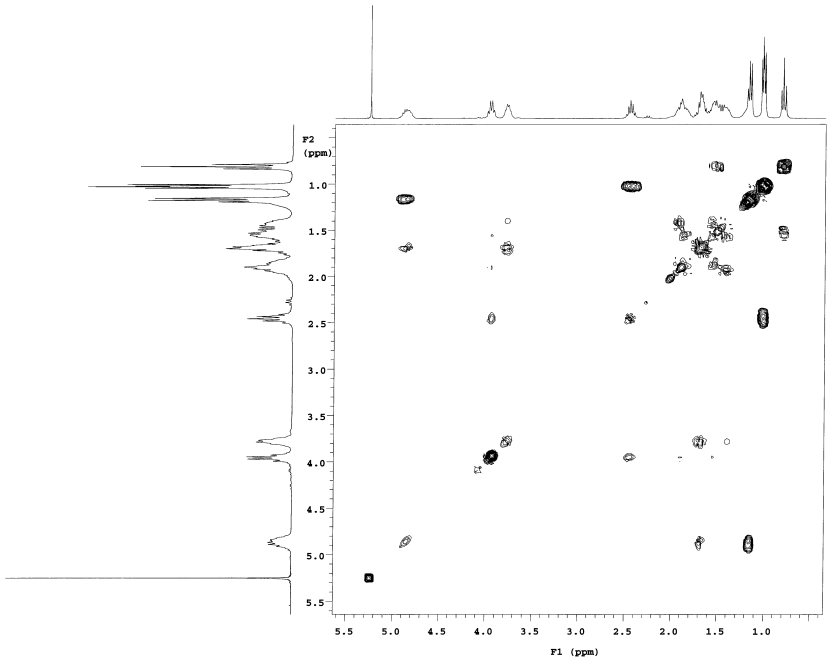


Figure 115. H,H COSY spectrum (300 MHz) of dinactin (**91**) in $CDCl_3$.

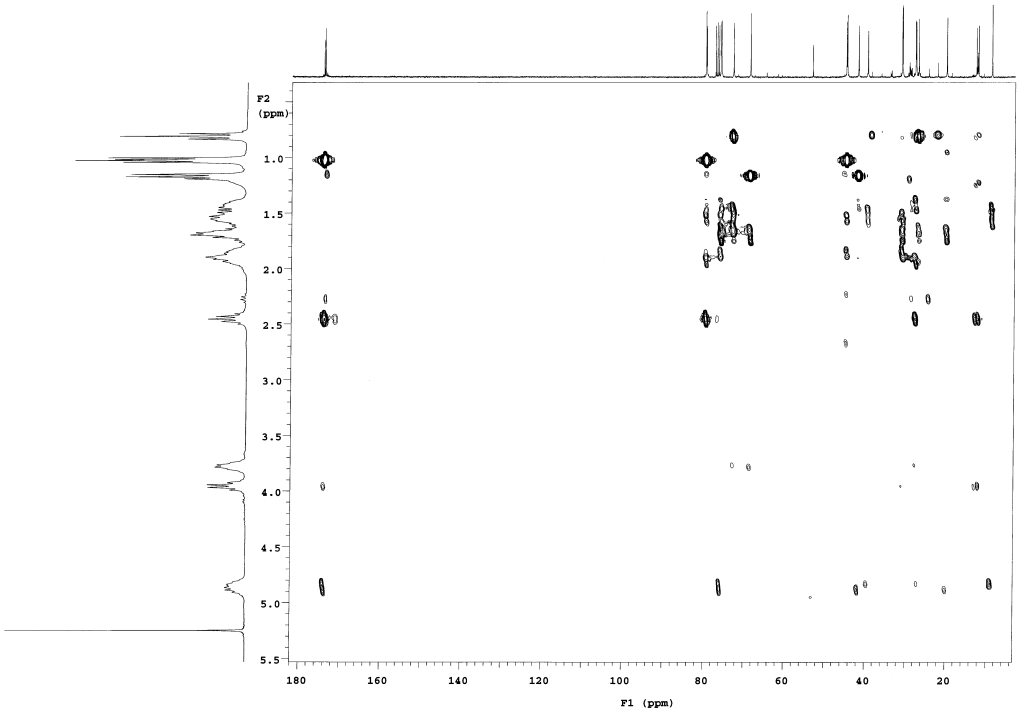
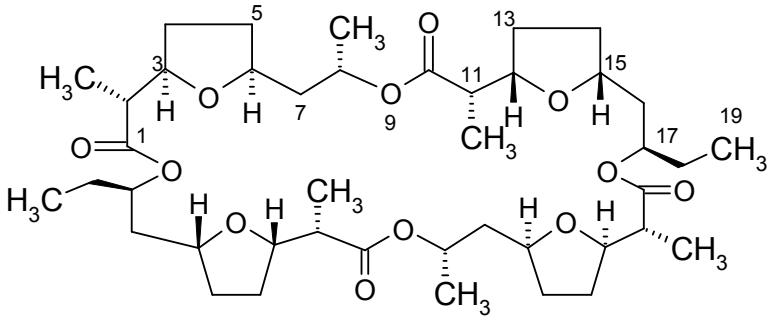


Figure 116. HMBC spectrum (300 MHz) of dinactin (**91**) in $CDCl_3$.



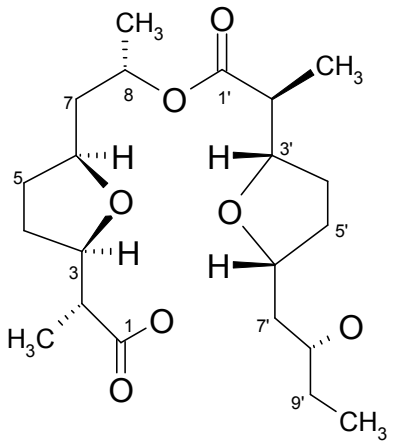
91

Dinactin (**91**) is a member of the macrotetrolide complex isolated from different *Streptomyces* species. It is a monovalent cation ionophore with high selectivity for ammonium and potassium, and inhibits T-cell proliferation, which is induced by IL-2 and cytokine production at nanomolar levels for IL-2, IL-4, IL-5 and interferon-γ.[169,170] Dinactin has antibacterial activity against *Staphylococcus aureus* 209P and *Mycobaterium bovis* strain B. C. G[171] as well as insecticidal activity.[172]

6.2.2 Bonactin

As a result of the separation of fraction III (Figure 112), a slightly polar band showed a violet colour by anisaldehyde/sulphuric acid reaction and afforded a brown oil. The ^{1}H NMR spectrum of compound **92** showed close structural similarities to dinactin (**91**). It displayed three multiplets for six oxygenated methine at δ 4.18 (H-6'), 3.98 (H-3,3',6) and 3.75 (H-8'), as well as to a 2H multiplet at δ 2.50 (H-2,2') of two methine protons linked to sp^2 carbon. Furthermore, a multiplet of two methylene groups was found in the range of δ 2.08-1.93 (H-5,4). Finally, the 1H NMR spectrum exhibited three doublets and one triplet each of 3H of methyl groups at 1.24 (CH_3-8), 1.17 (CH_3-2), 1.13 (CH_3-2') and 0.96 (CH3-10'), of which the last one was assigned to a terminal ethyl group.

The ESI mass spectrum showed a molecular ion peak at *m/z* 423 $[M + Na]^+$ affording a molecular weight of 400 Dalton. Searching in AntiBase using the above ^{1}H NMR and the molecular mass resulted in bonactin (**92**), which was further confirmed by direct comparison with the literature.[167]



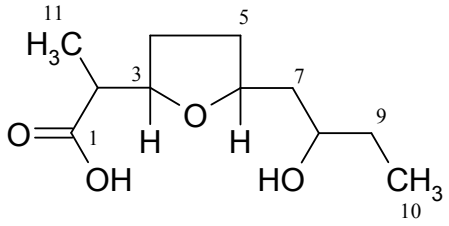
92

Bonactin (**92**) is a 15-membered macrodiolide isolated from the culture broth of *Streptomyces griseus* (strain GT051022). It was also a metabolite of *Streptomyces* sp. 6167 of marine origin.[173] Bonactin (**92**) has the same activity as nonactic acid (**88**), it showed moderate inhibition of the 3α–hydroxysteroid dehydrogenase, moderate cytotoxicity against L-929, K562 and HeLa cell line, and exhibit 50% inhibition of Coxackie virus B3 at 25 mg/mL. Bonactin (**92**) was synthesized by Lee and co-worker.[174, 175]

6.2.3 (+)-Homononactic acid

Fraction IV showed a non-polar band, characterized by a violet colour reaction with anisaldehyde/sulphuric acid. Further purification starting with PTLC followed by Sephadex LH-20 led to a colourless oil. The ^{1}H NMR spectrum showed a close similarity with nonactic acid (**88**). The main difference was the displacement of the methyl doublet in nonactic acid by a methyl triplet in (+)-homononactic acid, as the latter has one methylene group more.

ESIMS of this compound delivered a molecular ion peak 239 for $[M + Na]^+$ and the molecular weight was deduced to be 216 Dalton, which is *Δm* 14 units higher than that of nonactic acid. The structure was confirmed by comparison of the ^{1}H NMR data with literature values. (+)-homononactic acid (**93**) and its triple epimer at the carbon atoms C-2/C-3/C-6 was synthesized by Sharma *et al*.[176]



93

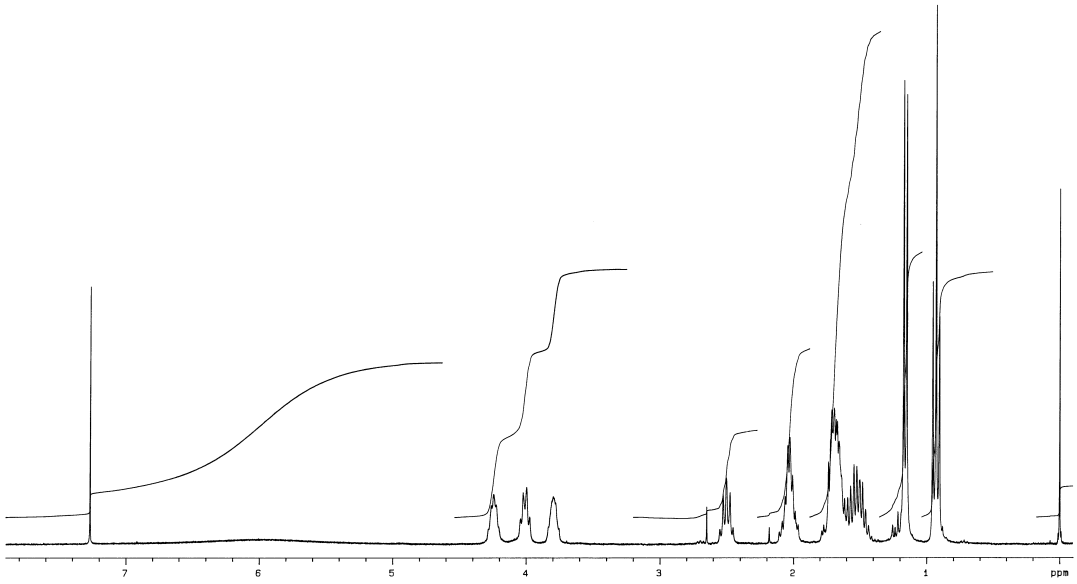


Figure 117. ^{1}HNMR spectrum (300 MHz) of (+)-homononactic acid (**93**) in $CDCl_3$.

6.3 Terrestrial *Streptomyces* Wo 990

The culture of the terrestrial *Streptomyce*s sp. Wo 990 in M_2 medium at 28 °C in a 25-liter shaker culture scale delivered a crude extract, which in the biological pre-screening exhibited high activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes*, *Mucor miehei* and *Chlorella sorokiniana*. The chemical screening using TLC showed two interesting spots, which turned greenish-blue, and brownish-green respectively with anisaldehyde/sulphuric acid.

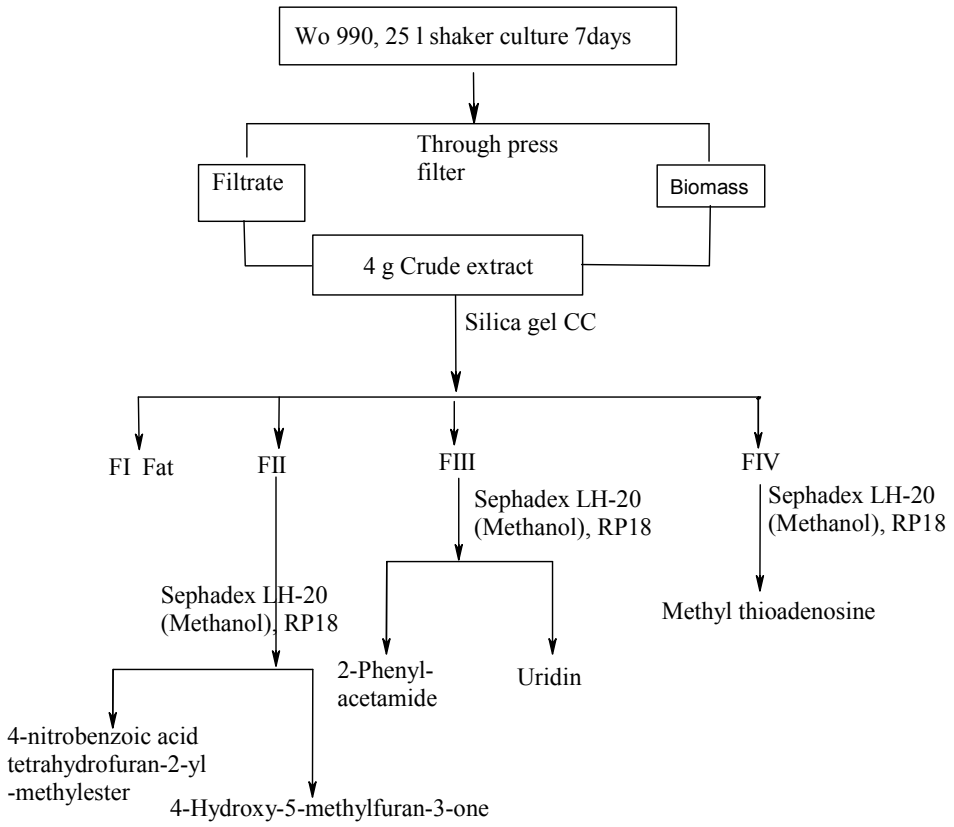


Figure 118. Work up scheme of the terrestrial *Streptomyces* sp. Wo 990.

6.3.1 4-Nitrobenzoic acid tetrahydrofuran-2-yl-methylester

Compound **94** was isolated from fraction II as an UV absorbing (254 nm), blue fluorescent (366 nm) colourless solid, which turned to greenish-blue on spraying with anisaldehyde/sulphuric acid reagent. ESI MS of compound **94** indicated a molecular weight of *m/z* 251, and ESIHRMS confirmed the molecular formula as $C_{12}H_{13}NO_5$ entailing seven double bond equivalents. The UV spectra of **94** displayed two strong bands at λ_{max} 320 and 380 nm. ^{1}H and ^{13}C NMR data were listed in Table 11.

The ^{1}H NMR spectrum exhibited doublets of two *o*-coupled proton signals each of 2H at δ 8.32 (d, ^{3}J = 8.9 Hz H-4,4) and 8.24 (d, ^{3}J = 8.98 Hz, H-3,7) in the aromatic region, indicating the presence of 1,4-disubistituted benzene ring. In the aliphatic region, there were multiples of two oxy-methylene groups CH_2-1' (δ 4.31, 4.39) and CH_2-5' at δ 3.87 as well as one oxy-methine group at δ 4.27. Additionally, multiplets of two methylene signals CH_2-4' (δ 1.93) and CH_2-3' (δ 1.76, 2.09) were observed.

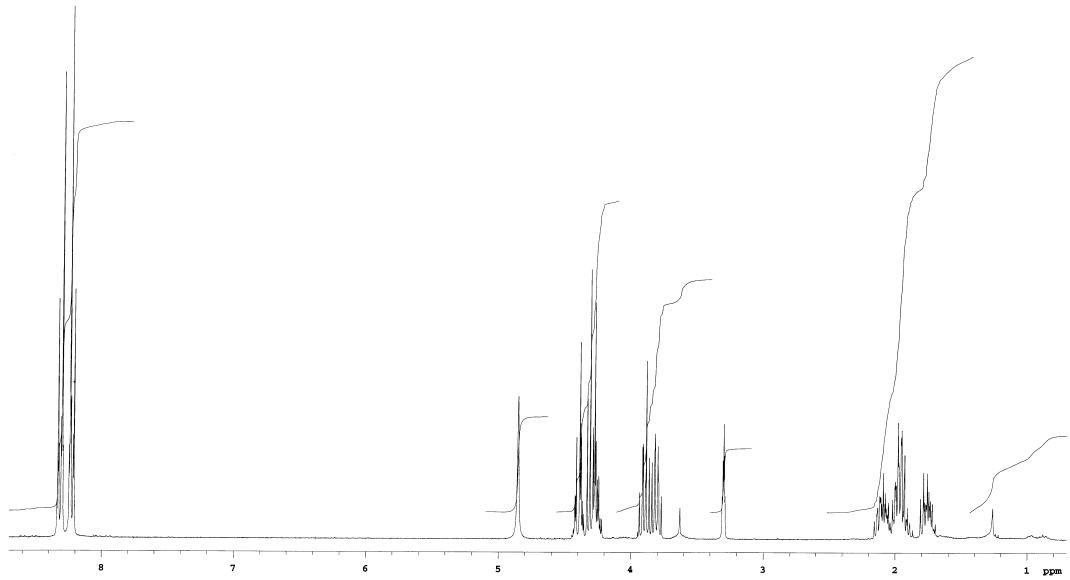


Figure 119. H,H COSY spectrum (300 MHz, CD_3OD) of 4-Nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**).

The ^{13}C NMR and HMQC spectra of **94** indicated the presence of 12 carbon signals, of which a carbonyl group appeared at δ 165.9. In the aromatic region four carbon signals of the benzene ring were observed at δ 152.1 (C-5), 136.8 (C-2), 131.9 (C-4,6) and 124 (C-3,7) respectively. On the other hand, the sp^3 region displayed signals of oxy-carbon signals at δ 68.5 (C-1'), 77.9 (C-2') and 69.5 (C-5').

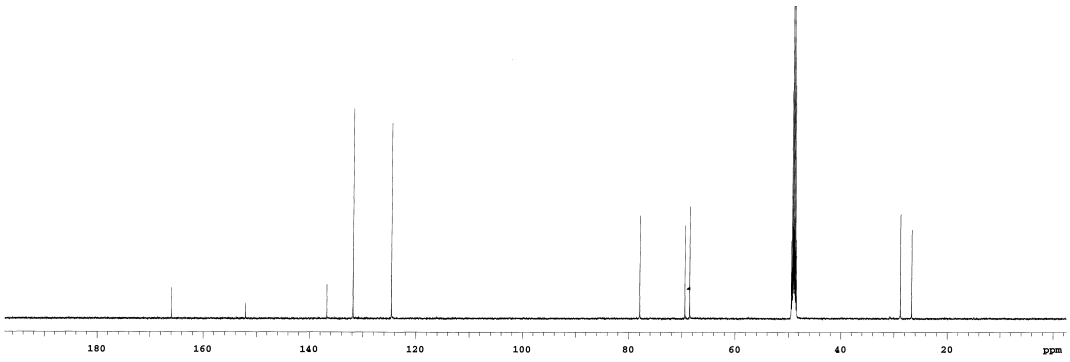
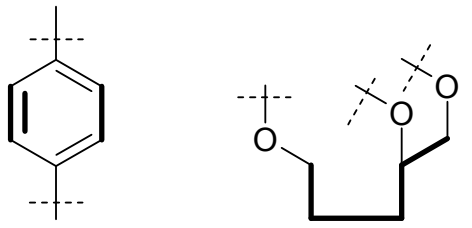


Figure 120. H,H COSY spectrum (125 MHz, CD_3OD) of 4-Nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**).

From the revealed spectroscopic data, there were two possible structures, 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**) and 4-nitrobenzoic acid tetrahydropyran-3-yl-ester (**95**), so the sample was subjected to 2D experiment. Inspection of the ^1H NMR spectrum and H,H COSY NMR data, the isolated compound was confirmed as compound **94**, which was composed of 1,4-disubstituted aromatic ring fraction A. Furthermore, in the H,H COSY spectrum the signals of the oxygenated me-

thylene CH_2-2' showed strong correlation to the methine CH-1' and the methylene CH_2-3'. Finally the methylene CH_2-4' showed a strong correlation to the methylenes CH_2-5' and CH_2-3', which led to 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**).



A **B**

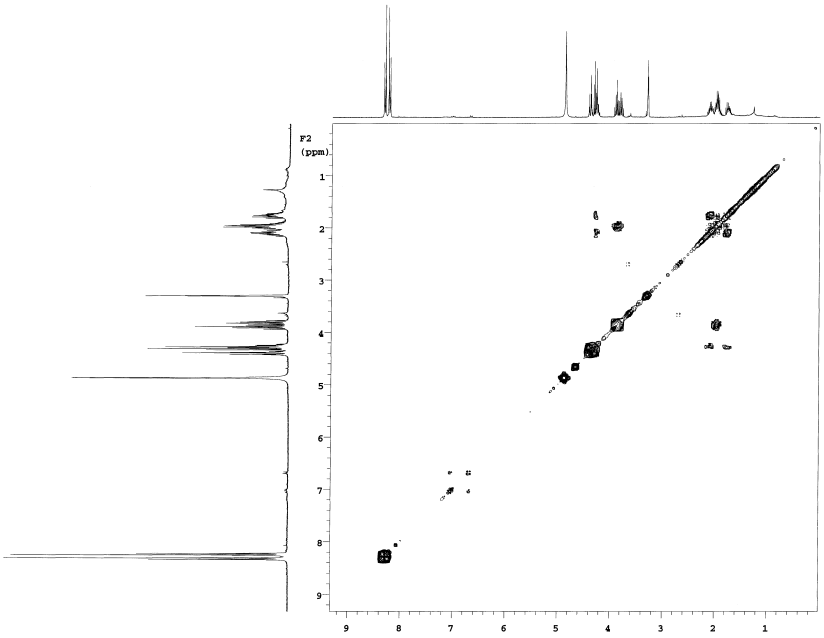
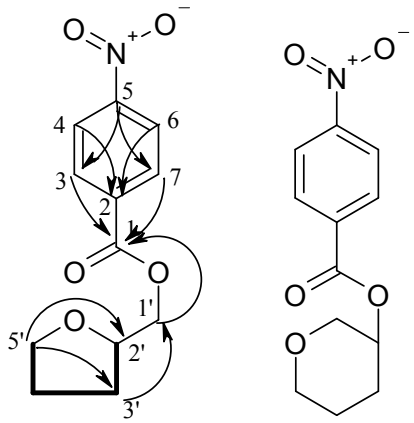


Figure 121. H,H COSY spectrum (300 MHz, CD_3OD) of 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**).

From the HMBC measurement, a ^3J correlation from proton signals of CH-3,7 at δ 8.24 and the methylene group CH_2-1' at (δ 4.39, 3.31) to the carbonyl carbon CO-1 (δ 165.9) was observed, which connected sub-structures A and B.



94 **95**

Figure 122. Selected H,H COSY (▬) and HMBC (→) correlations of compound **94**.

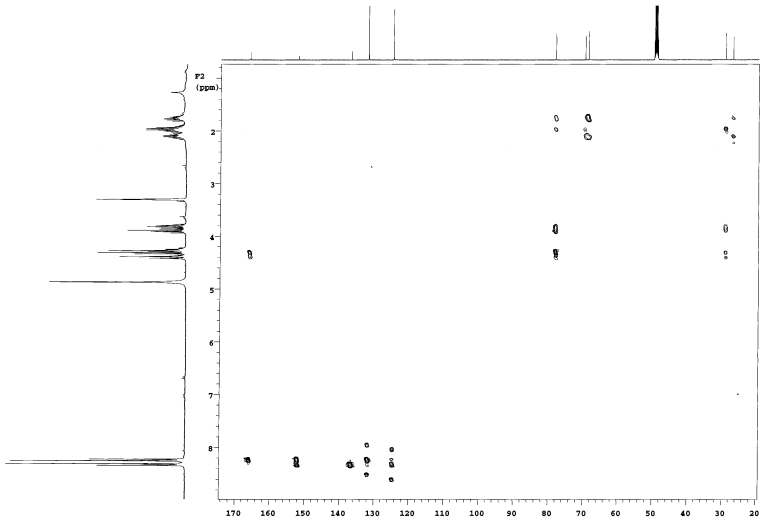
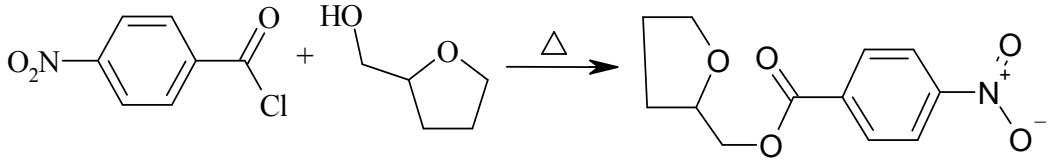


Figure 123. HMBC spectrum (300 MHz, CD_3OD) of 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**).

On the other hand, structure **94** was further confirmed synthetically by reaction of 4-nitrobenzoylchloride (**96**) with furfuryl alcohol (**97**) and the reaction was done by Dr. Sayed from Egypt. The synthesized derivative gave the same ^{1}H NMR data, melting point, R_f value and finally the same colour reaction with anisaldehyde/sulphuric acid, so that the isomer **95** was excluded.



96 **97** **94**

Figure 124. Synthesis of compound **94**.

Table 11. ^{1}H and ^{13}C NMR assignments of compound **94** (*J* in Hz).

No.	Compound **94**		No.	Compound **98**	
	δ_C	δ_H		δ_C	δ_H
1	165.9	-	2	73.0	4.49 (s)
2	136.8	-	3	196.2	-
3,7	124.6	8.24 (d, 8.9)	4	135.2	-
4,6	131.9	8.32 (d, 8.9)	5	176.2	-
5	152.1	-	6	13.6	2.42 (s)
1'	68.5	4.31, 439 (m)			
2'	77.9	4.27 (m)			
3'	28.8	1.76, 2.09 (m)			
4'	26.7	1.93 (m)			
5'	69.5	3.87 (m)			

Compound **94** was found to inhibit in the agar diffusion test the growth of the micro-algae *Chlorella vulgaris* (23 mm), *Chlorella sorokiniana* (34 mm), *Scenedesmus subspicatus* (30 mm) and it showed also moderate (54%) cytotoxicity against brine shrimp.

6.3.2 4-Hydroxy-5-methylfuran-3-one

Compound **98** was obtained together with **94** from the same fraction as a colourless solid, which showed a brownish green colour with anisaldehyde/sulphuric acid. The molecular weight was deduced by EIMS as *m/z* 114 and the molecular formula of **98** was determined to be $C_5H_6O_3$ by HREIMS. The ^{1}H and ^{13}C NMR data of **98** are listed in Table 11. The ^{1}H NMR spectrum in CD_3OD showed a singlet at δ 4.49 of the oxygenated methylene group and another singlet at δ 2.42 due to a methyl group.

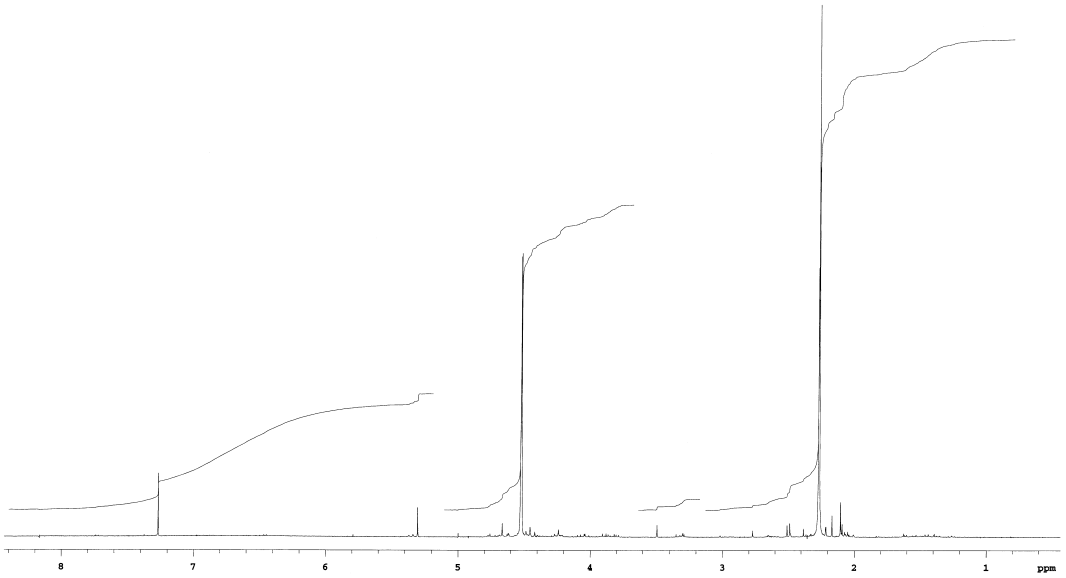


Figure 125. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 4-hydroxy-5-methylfuran-3-one (**98**).

The ^{13}C NMR and HMQC spectra of **98** revealed the presence of five carbon signals, a carbonyl group at δ 196.2, two sp^2 carbon signals at δ 135.2 (C-4) and δ 176.2 (C-5) due to an α,β-unsaturated carbonyl. In addition, two sp^3 carbon signals due to an oxygenated methylene carbon at δ 73.0 and a methyl group at δ 13.6 were observed.

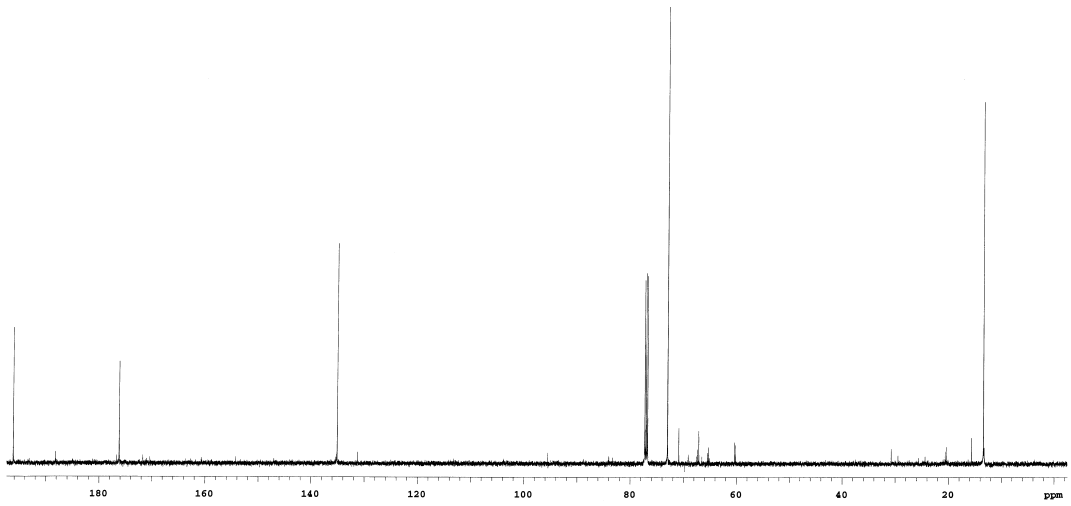
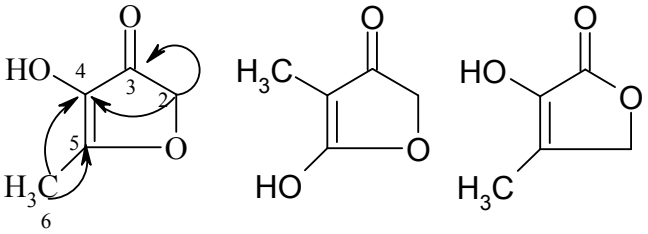


Figure 126. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 4-hydroxy-5-methylfuran-3-one (**98**).

With the above spectroscopic data, three structures are possible, 4-hydroxy-5-methylfuran-3-one (**98**), 5-hydroxy-4-methyl-furan-3-one (**99**), and 3-hydroxy-4-methyl-5*H*-furan-2-one (**100**). To determine the final structure, 2D experiments were performed. In the HMBC spectrum, a strong correlation from the methylene group at δ 4.49 to the carbonyl carbon at 196.2 (CO-3) and the oxygenated sp^2 carbon at 135.2 (C-4) was observed. The methyl protons revealed strong correlations to C-5 at 176.2 and C-4. Thus, compound **99** was excluded. On the other hand, the high value of the carbonyl carbon signal (196.2) indicated a ketonic carbon, and not an acid derivative, and so compound **100** was also excluded. Both compounds **94** and **98** were synthetically well known.[177-179]



98 **99** **100**

Figure 127. Selected HMBC (→) correlations of 4-hydroxy-5-methylfuran-3-one (**98**).

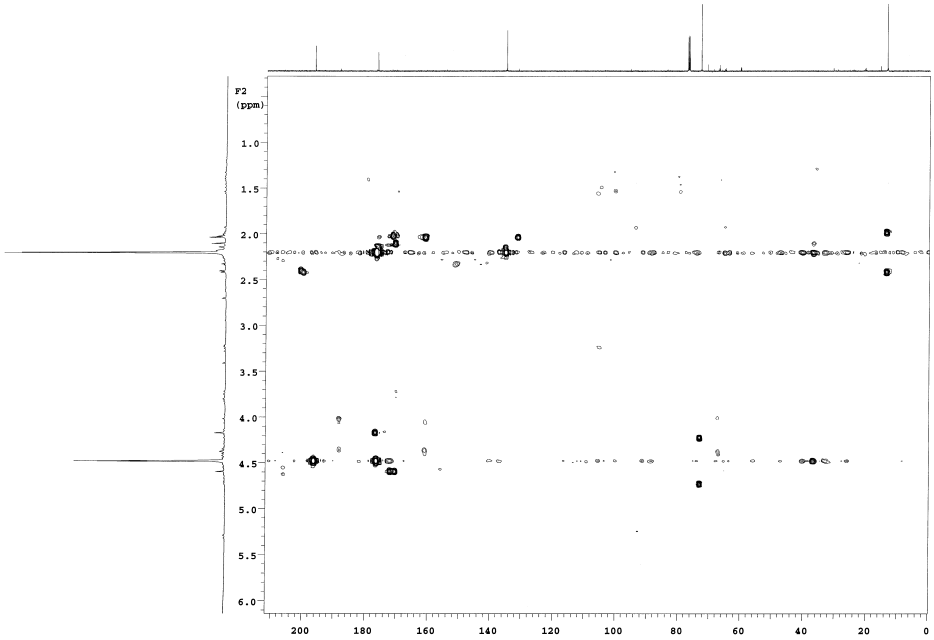


Figure 128. HMBC NMR spectrum (300 MHz, $CDCl_3$) of 4-hydroxy-5-methylfuran-3-one (**98**).

6.4 Terrestrial *Streptomyces* sp. Ank 150

The crude extract of the terrestrial *Streptomyces* sp. Ank 150 exhibited activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57), *Mucor miehei* and *Candida albicans*. In the chemical screening the extract showed fluorescent (366 nm) and UV absorbing (254 nm) bands, which turned light green on spraying with anisaldehyde/sulphuric acid reagent, while others turned red.

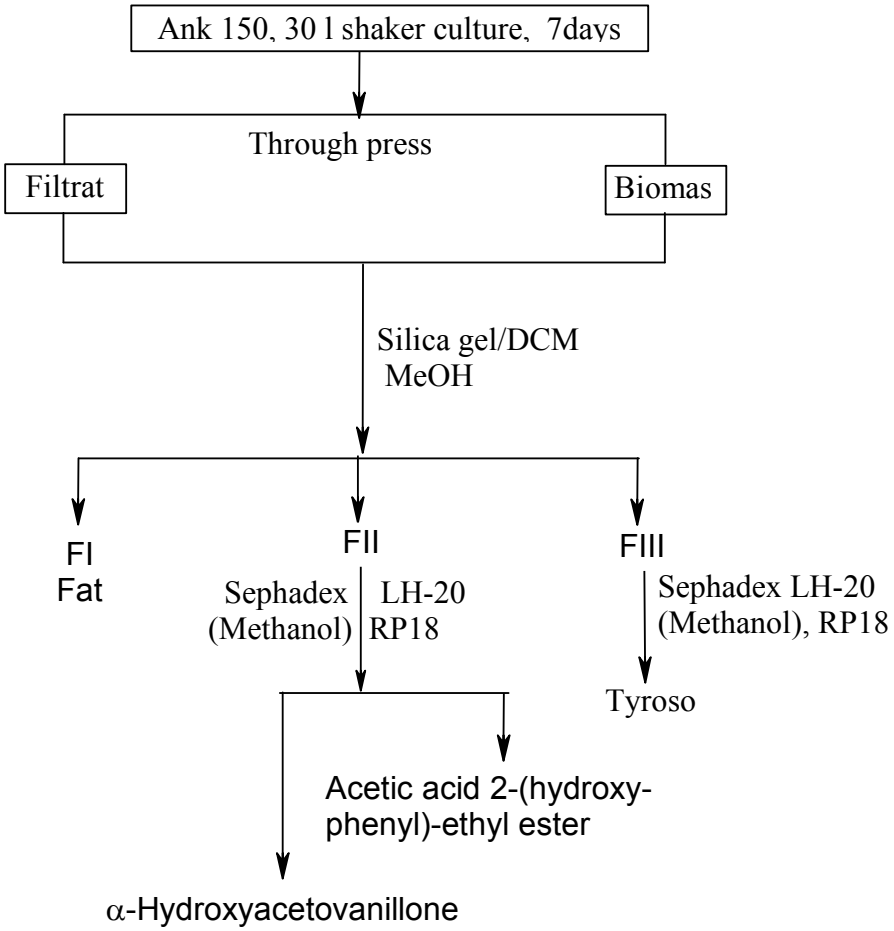


Figure 129. Work-up scheme of the Marine *Streptomyces* sp. Ank 150

6.4.1 α-Hydroxyacetovanillone

Compound **101** was isolated as UV absorbing (254 nm), blue fluorescent (366 nm) colourless solid, which turned to greenish-blue on spraying with anisaldehyde/sulphuric acid reagent. ESIMS of compound **101** indicated a molecular weight of *m/z* 182, and ESI HRMS confirmed the molecular formula as $C_9H_{10}O_4$ entailing five double bond equivalents.

The UV spectra **101** displayed four strong bands at λ_{max} 204, 229, 277 and 302 nm in neutral solution. Under basic conditions, the latter band showed a bathochromic shift to λ_{max} 344 nm. The ^1H and ^{13}C NMR data were listed in Table 12. The ^1H NMR spectrum in CD_3OD exhibited the typical signals of a 1,2,4-trisubistituted benzene ring. In the high-field region, it showed a resonance of two oxygenated groups, one of 2H for an oxy-methylene at δ 4.83, the other of 3H for the methoxy group at δ 3.90. Furthermore, the ^1H NMR spectrum displayed two exchangeable proton signals due to two OH groups at δ 3.54 as a triplet and a broad singlet at δ 6.14.

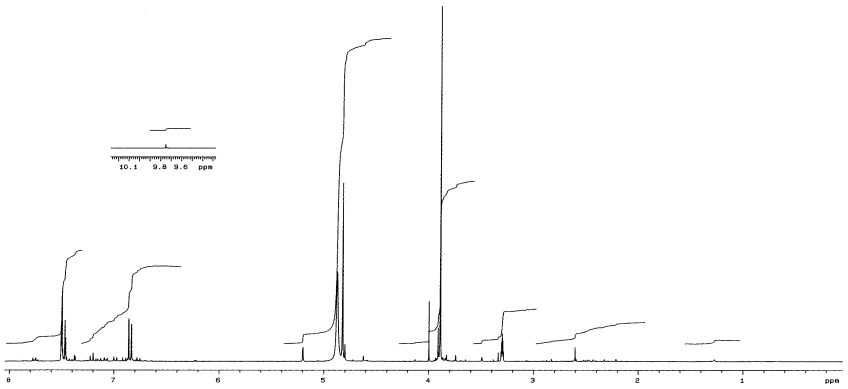


Figure 130. ^{1}H NMR spectrum (CD_3OD, 300 MHz) of α-hydroxyacetovanillone (**101**).

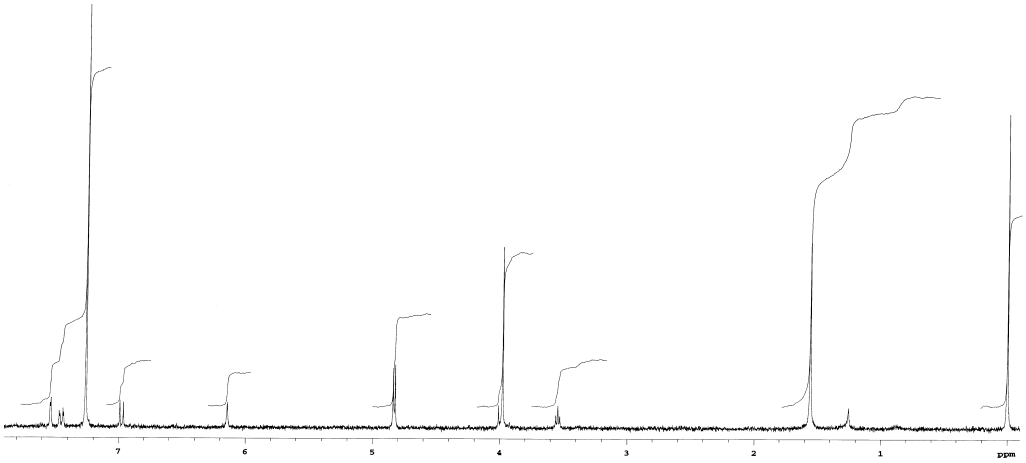


Figure 131. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of α-hydroxyacetovanillone (**101**).

The ^{13}C NMR and HMQC spectra of **101** indicated 9 carbon signals, a carbonyl group at δ 198.5, six sp^2 carbons of the benzene ring, one oxy-methylene as well as a methoxy group.

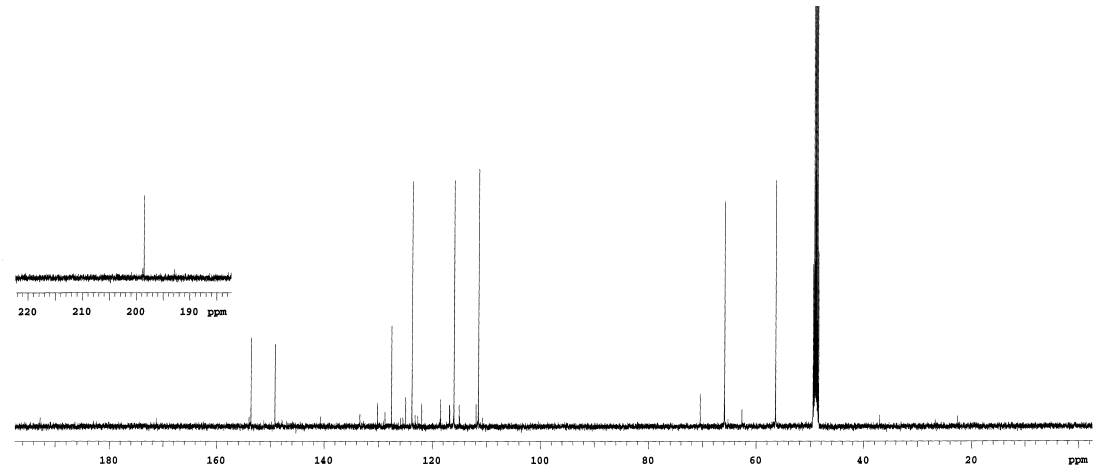
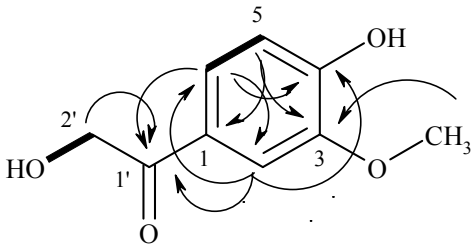


Figure 132. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of α-hydroxyacetovanillone (**101**).

The HMBC spectrum showed a ^{3}J correlation from the methoxy group (δ 3.90) to carbon-3 (δ 149.5), which located the latter at C-3, and also ^{3}J correlations were observed from the doublet of H-5 (δ_H 6.85) to C-3 (δ 149.5) and C-1 (δ 127.2). Based on the ^{3}J correlation from H-2 (δ 7.52) and H-6 (δ 7.50) to the carbonyl carbon (δ 198.5) the carbonyl was located at C-1, resulting in α-hydroxyacetovanillone (**101**). α-Hydroxyacetovanillone derivatives have been described as wound-inducible agents that activate virulence genes of *Agrobacterium tumefaciens*, especially in species within the *Solanaceae* family.[180]



101

Figure 133. Selected HMBC (→) and H,H COSY (▬) correlations of α-hydroxyacetovanillone (**101**).

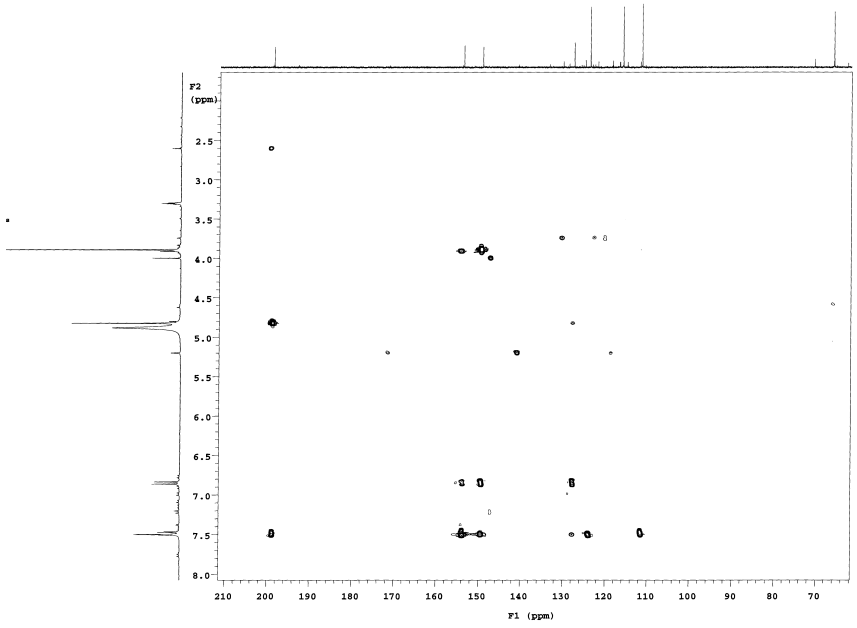


Figure 134. HMBC spectrum (CD_3OD, 500 MHz) of α-hydroxyacetovanillone (**101**).

Table 12. ^{1}H and ^{13}C NMR assignment of compound **101** (*J* in Hz).

Position	δ_C [a,b]	δ_H [a,c]	δ_H [c,d]
1	127.2	-	-
2	111.4	7.52 (d, 2.0)	7.54 (d, 2.1)
3	149.5	-	-
3-OCH_3	56.4	3.90 (s)	3.98 (s)
4	154.6	-	-
4-OCH_3	-	-	-
4-OH	-	-	6.14 (brs)
5	116.2	6.85 (d, 8.2)	6.98 (d, 8.2)
6	124.0	7.50(dd, 8.2,	7.45 (dd, 8.3,
1'	198.5	-	-
2'	65.8	4.83 (s)	4.83 (d, 4.7)
2'-OH	-	-	3.54 (t, 4.6)

[a] CD_3OD, [b] 125 MHz; [c] 300 MHz; [d] $CDCl_3$.

6.4.2 Acetic acid 2-(4-hydroxy-phenyl)-ethyl ester

Compound **102** was obtained as colourless solid from fraction III after further fractionation on Sephadex LH-20 and final purification on RP-18. It showed a reddish-brown colouration when exposed to anisaldehyde/sulphuric acid and heating. The molecular weight was deduced by ESIMS as *m/z* 180, corresponding to the molecular formula of $C_{10}H_{12}O_3$.

The ^{1}H NMR spectrum displayed in the aromatic region two *o*-coupled proton signals at δ 7.03 and 6.70 of 1,4-disubstituted benzene ring. In the aliphatic region a resonance of two methylene triplets at δ 4.18 and 2.81 were seen. Additionally, an acetyl singlet was observed at δ 1.96.

The ^{13}C NMR and HMQC spectra delivered signals for one carbonyl group at δ 172.9, six benzene carbons in the range of δ 157.1-116.2 of which one at δ 157.1 was oxygenated, an oxy-methylene at δ 66.6, further CH_2 group at δ 35.2 along with a methyl carbon at δ 20.8. According to the above spectroscopic data, two structures were possible, acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**102**) and acetic acid 4-(2-hydroxy-ethyl)-phenyl ester (**103**).

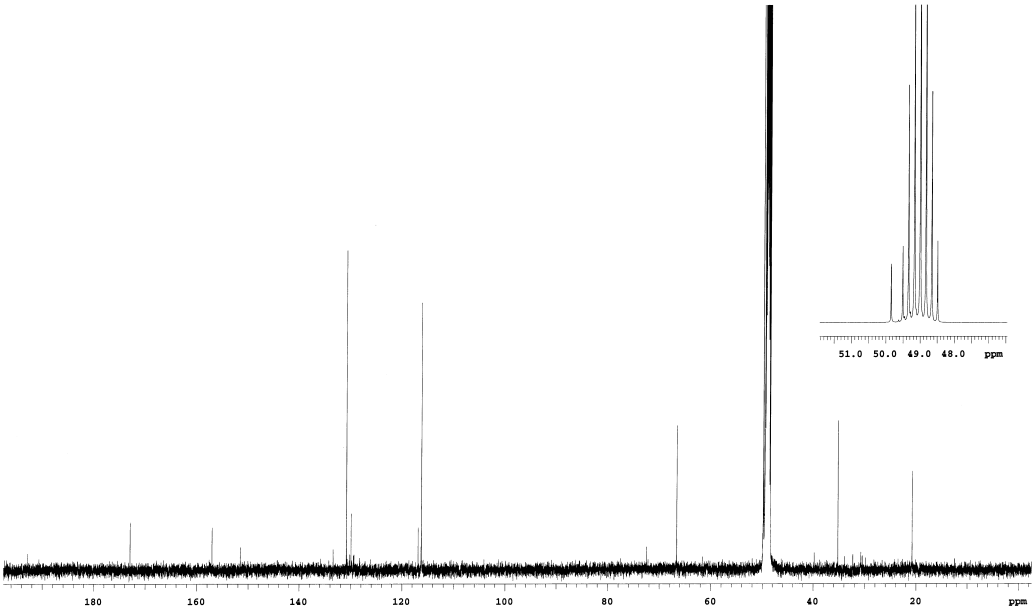


Figure 135. ^{13}C NMR spectrum (CD_3OD, 500 MHz) of acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**102**).

Due to the ^{3}J correlation from CH_2-2' to the carbonyl signal C-4' (172.9) which observed in the HMBC spectrum structure **103** was excluded and acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**102**) confirmed.

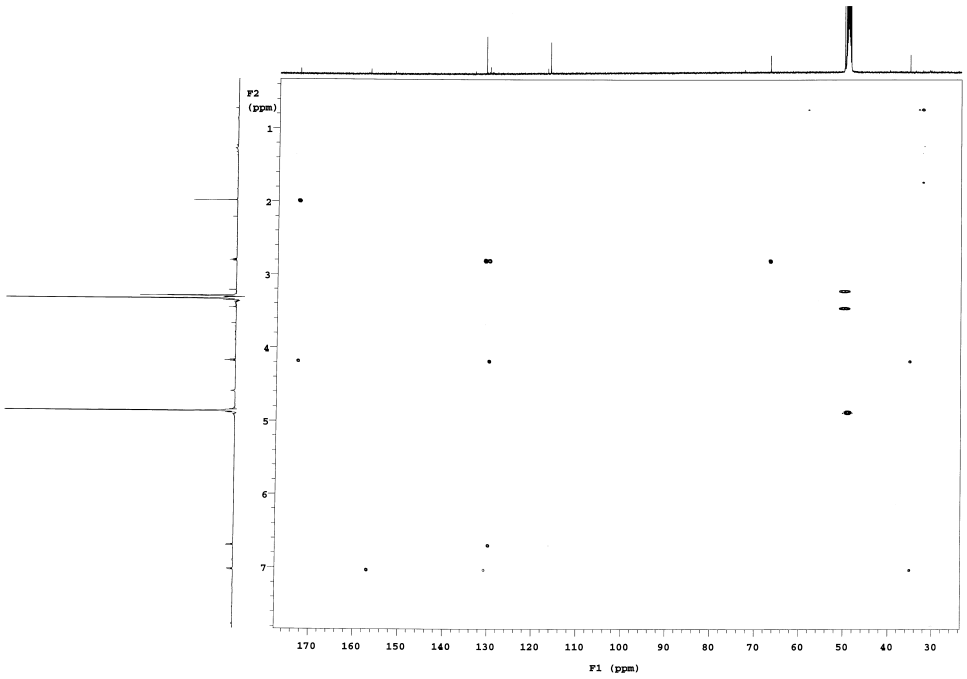
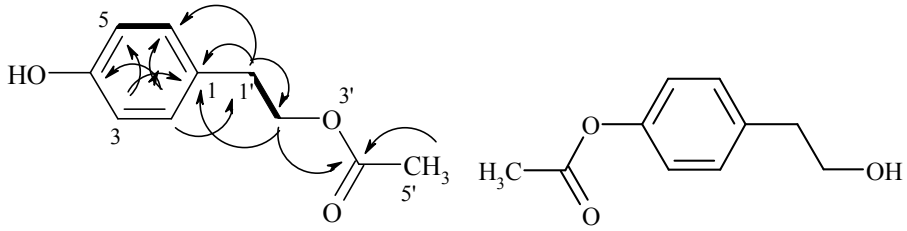


Figure 136. HMBC spectrum (CD_3OD, 125 MHz) of acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**102**).



102 **103**

Figure 137. Selected HMBC (→) and H,H COSY (▬) correlations of acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**102**).

Table 13. ^{1}H and ^{13}C NMR assignments of **102** in CD_3OD (*J* in Hz).

No.	Compound **102**	
	δ_C [a]	δ_H [b], ^{3}J
1	129.9	-
2, 6	130.9	7.03 (d, 8.5)
3, 5	116.2	6.70 (d, 8.5)
4	157.1	-
1'	35.2	2.81 (t, 7.0)
2'	66.6	4.18 (t, 7.1)
4'	172.9	-
5'	20.8	1.96 (s)

[a] 125 MHz; [b] 600 MHz;

6.5 Terrestrial Streptomyces sp. Ank 192

The crude extract of the terrestrial Streptomyces sp. Ank 192 exhibited activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, but no activity against *Streptomyces viridochromogenes* (Tü57), *Mucor miehei* and *Candida albicans*. In the chemical pre-screening the extract showed UV absorbing (254 nm) bands, which stained to blue, green and red colour on spraying with anisaldehyde/sulphuric acid reagent.

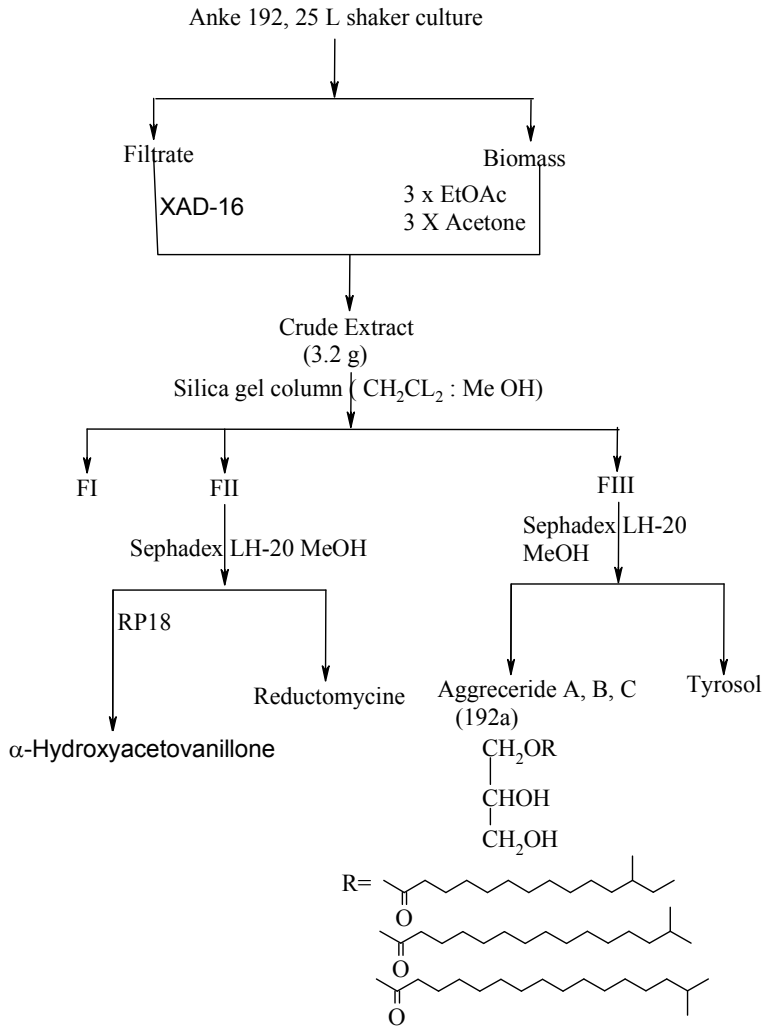


Figure 138. Work-up scheme of terrestrial *Streptomyces* sp. Ank 192.

6.5.1 Reductiomycin

Compound **104** was isolated as yellow needles from a UV absorbing band (254 nm), which stained to dark green with anisaldehyde-sulphuric acid. The ^{1}H NMR spectrum showed two broad singlets of H/D exchangeable protons at δ 13.76 and 7.68 due to OH and NH respectively. In the olefinic region one singlet at δ 6.90 (H-5') was observed and the two doublets at δ 7.50 (^3J = 15.1 Hz, H-3") and δ 5.81 (^3J = 15.1 Hz, H-2") were due to a *trans*-disubstituted α,β-unsaturated carbonyl derivative. In addition the ^{1}H NMR spectrum showed one acetal methine at δ 6.70 (H-2'), and an oxygenated methylene at δ 3.05. The methyl singlet at δ 2.10 indicated a connection with an sp^2 carbon atom.

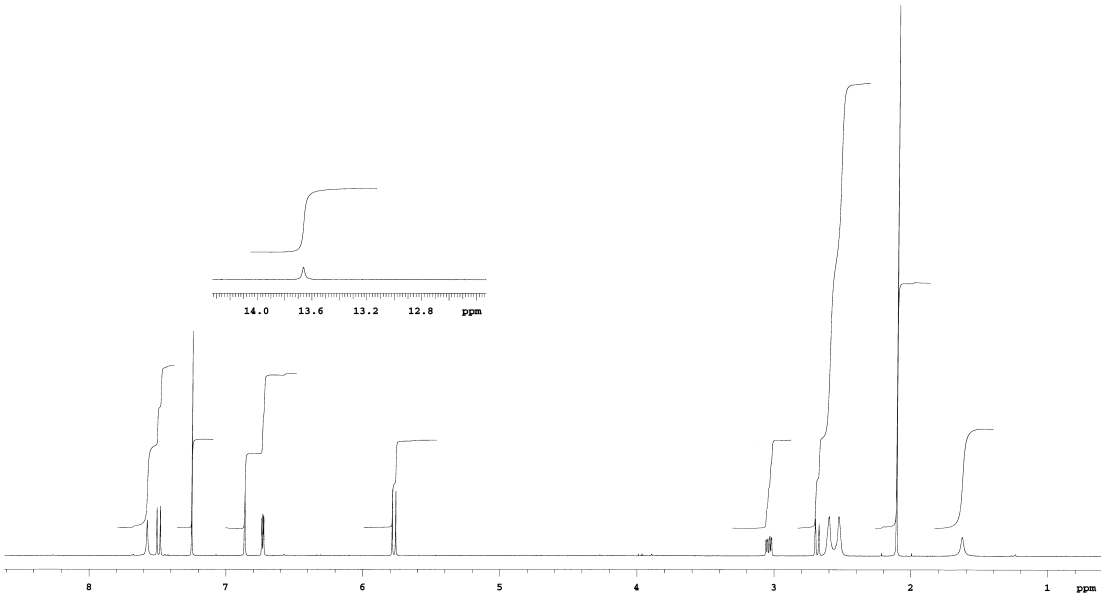
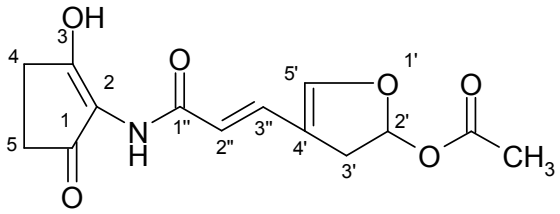


Figure 139. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of reductiomycin (**104**).

The molecular weight of **104** was deduced by EIMS to be 293, the odd molecular weight indicated the presence of odd number of nitrogen. A search in AntiBase using the above spectroscopic data as well as the molecular weight resulted in reductiomycin (**104**), which has been further confirmed by comparing with authentic spectra as well as literature data. [181]



104

Reductiomycin (**104**) is an antibiotic produced by several strains of streptomycetes, like *Streptomyces griseorubiginosus*,[182] *Streptomyces orientalis* or many marine isolates investigated in our group;[183] it has antitumor activity and weak activity against fungi and Gram-positive bacteria.[184]

6.6 Terrestrial *Streptomyces* sp. Ank22

The terrestrial *Streptomyces* sp. Ank22 was isolated by Prof. H. Anke from a soil sample. In our biological screening, the crude extract showed a good activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Streptomyces virido-*

chromogenes. The chemical screening indicated, in addition to an orange spot of actinomycin D, UV-absorbing bands, some of which turned violet and yellow-brown on spraying with anisaldehyde/sulphuric acid. The 25 L culture on M_2 medium delivered 2.5 g of a dark reddish-brown crude extract after standard work-up.

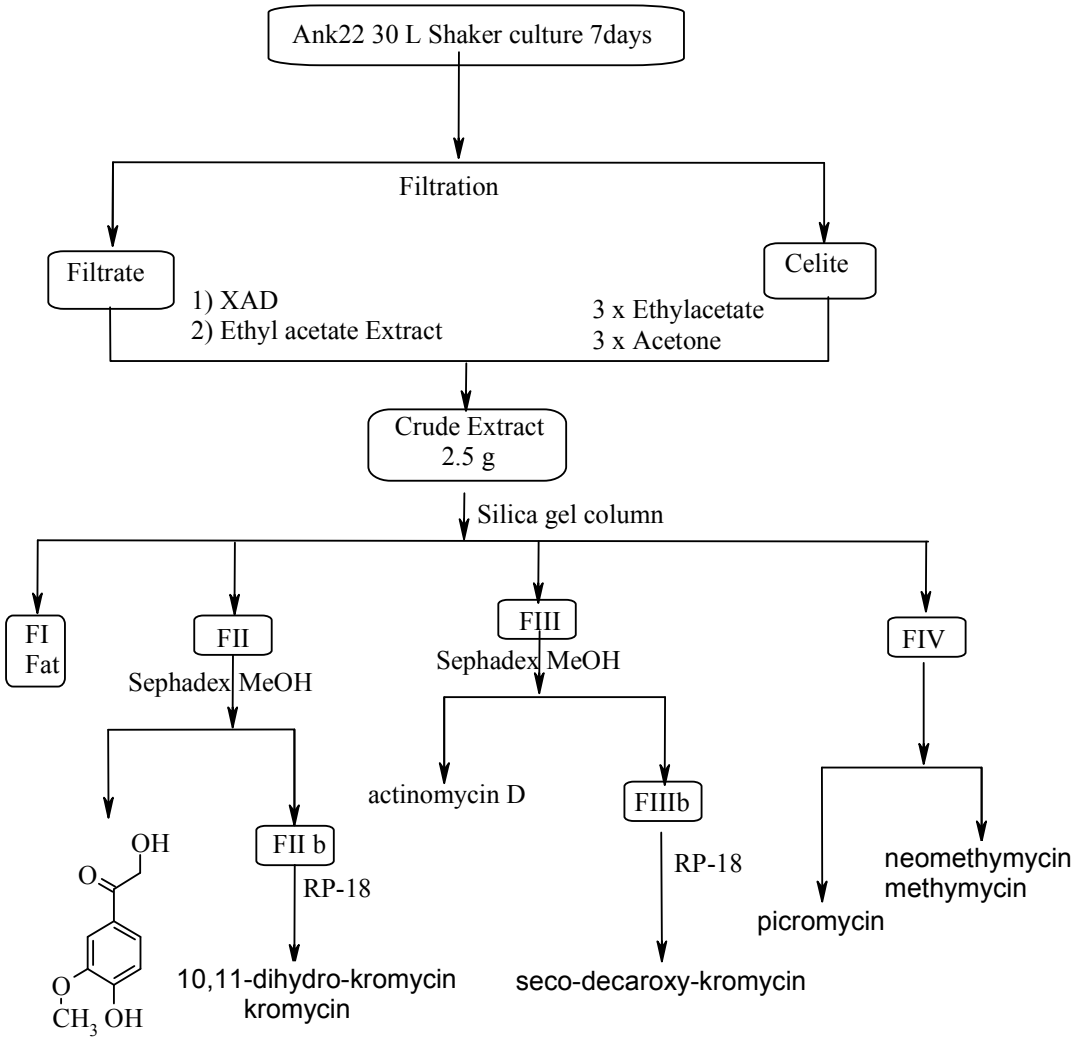


Figure 140. Work-up scheme of the terrestrial *Streptomyces* sp. Ank22.

6.6.1 Seco-Decarboxy-kromycin

TLC of fraction III (Figure 140) showed a UV active polar zone (R_f = 0.13 (CH_2Cl_2/5 % MeOH)), which gave a dark violet colour with anisaldehyde/sulphuric acid on heating. Fraction III was chromatographed on Sephadex LH-20 followed by a reverse phase C18 column to afford compound **105** as colourless oil. The molecular formula was deduced as $C_{19}H_{32}O_4$ by (+)-HRESIMS.

The ^{1}H NMR spectrum of **105** showed six methyl signals at δ 1.75 (d, CH_3-4), 1.24 (s, CH_3-12), 1.07 (d, CH_3-8), 1.04 (t, CH_3-1), 1.02 (d, CH_3-6), 0.98 (t, H-15). It

also displayed signals at δ 3.25 (H-13) attributed to one oxygen-bearing methine, another methine connected to sp^2 carbon at δ 2.84 (H-8), and partially overlapped multiplets between δ 2.69-1.44 due to three methylenes. Finally three olefinic proton signals at δ 6.98 (d, H-11), 6.35 (d, H-10) and 6.40 (dd, H-5), respectively, were observed, the first two displaying a *trans*-coupling.

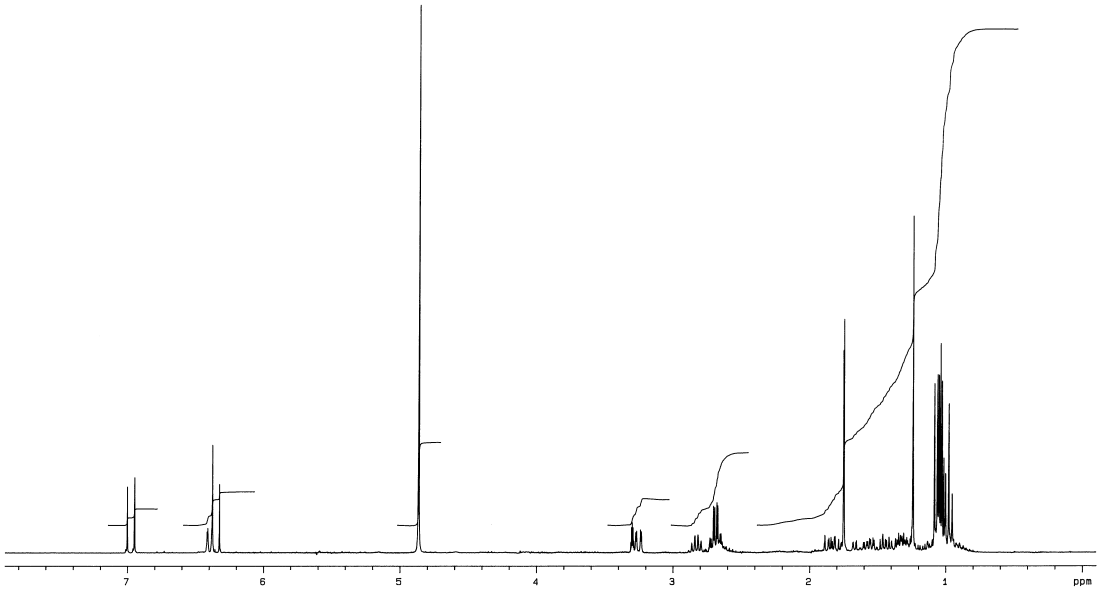


Figure 141. ^{1}H NMR spectrum (300MHz, CD_3OD) of *seco*-decarboxy-kromycin (**105**).

The ^{13}C NMR spectrum exhibited 19 carbon signals, among them two ketone carbonyls at δ 206.1 (CO-9) and 205.0 (CO-3) and two signals at δ 153.1 (CH-11) and 149.3 (CH-5) as expected for β-carbons of an α,β-unsaturated carbonyl system, where the α-carbons were observed at δ 136.1 (C-4) and 127.3 (C-10). In addition the spectrum also showed oxygenated carbon signals at δ 79.9 (CH-13) and 76.3 (C_q-12), two methine carbons at δ 43.6 (C-8), and 33.6 (C-6), three methylene carbons at δ 41.2 (C-7), 31.3 (C-2) and 25.4 (C-14). Furthermore the ^{13}C NMR spectrum revealed signals for six aliphatic methyl groups.

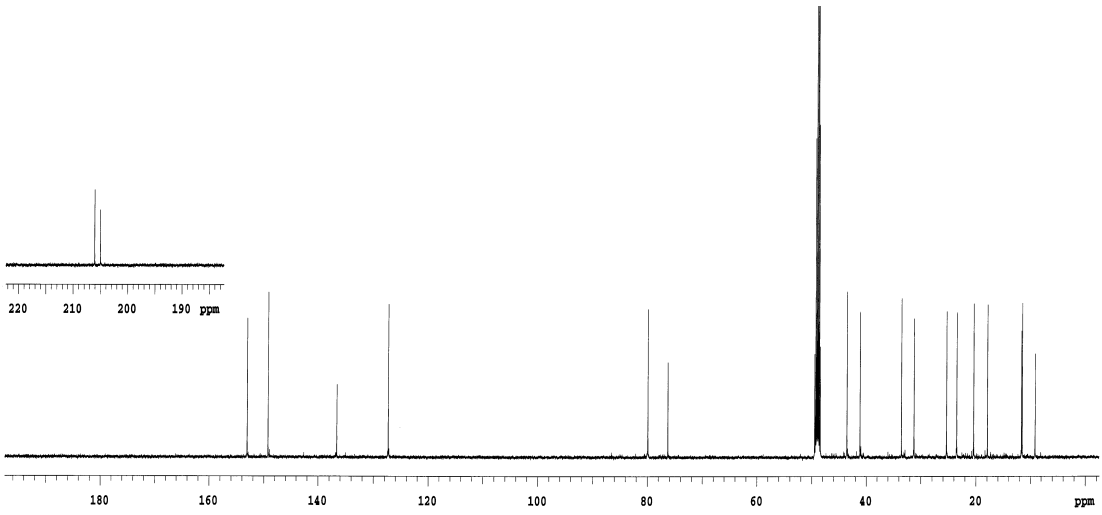


Figure 142. ^{13}C NMR spectrum (125 MHz, CD_3OD) of *seco*-decarboxy-kromycin (**105**).

From the H,H COSY spectrum it was clear that the two olefinic protons at δ 6.98 and 6.35 coupled with each other to yield fragment A. The methylene protons at δ 1.55 (H-14) showed ^3J correlation with the oxygenated methine at δ 3.25 and the methyl group at δ 0.98 to afford fragment B. The olefinic proton at δ 6.40 showed allyl coupling with the methyl group at δ 1.75, and the methine proton at δ 2.66 correlated with the methyl at δ 1.02 and the methylene at δ 1.84 and 1.44. The latter methylene showed ^3J coupling with the methine at δ 2.84, which coupled with the methyl at δ 1.07 to give fragment C. Finally the spectrum pointed to the presence of an ethyl group.

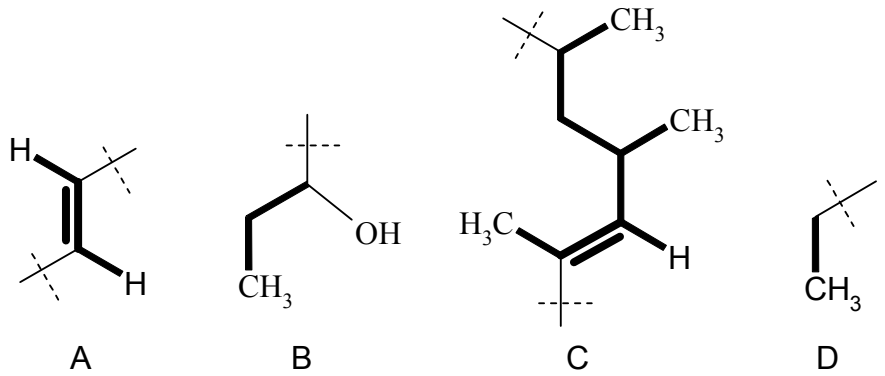


Figure 143. Fragments resulted from the 1H NMR and H,H COSY spectra of *seco*-decarboxy-kromycin (**105**).

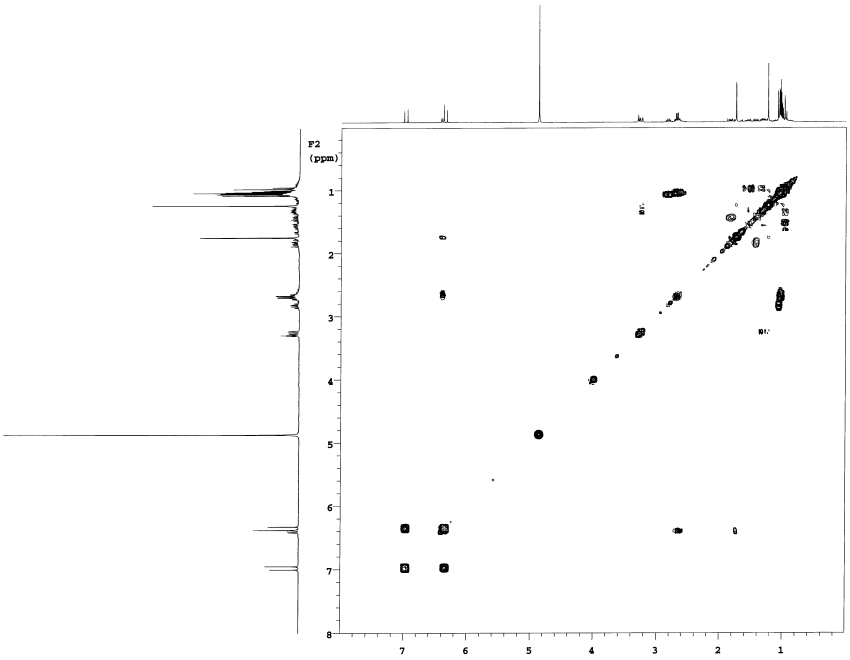
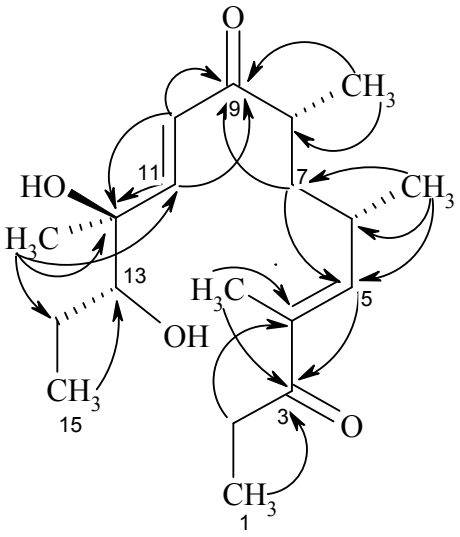


Figure 144. H,H COSY spectrum (300 MHz, CD_3OD) of *seco*-decarboxy-kromycin (**105**).

The HMBC correlations confirmed the presence of the α,β-unsaturated carbonyl system and connected fragments A and C. The HMBC correlation of the methyl protons CH_3-1 (δ 1.24), the methine H-13 (δ 3.25), the two olefinic signals of H-10 and H-11 to the oxygenated quaternary carbon C-12 (δ 76.3) indicated that the methyl group is located at C-12 and connected fragment A and B together. Finally, the attachment of the ethyl group to C-3 and the connection between fragment C and D was confirmed by HMBC correlation from the two methyl groups C-1 (1.04) and CH_3-4, the methylene H-2, the olefins proton H-5 (6.40) to the carbonyl CO-3. As a result, the final structure of **105** was obtained.



105

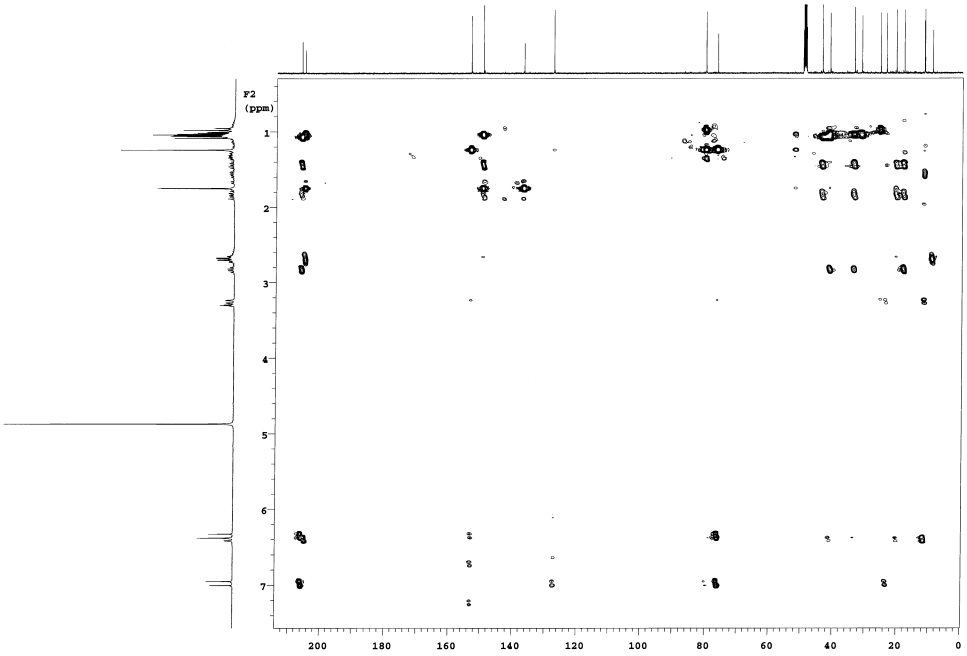


Figure 145. HMBC spectrum (300MHz, CD_3OD) of *seco*-decarboxy-kromycin (**105**).

Compound **105** is a new secondary metabolite, isolated in parallel within our group from the terresterial *Streptomyces* sp. Ank 26.

6.6.2 10,11-Dihydro-kromycin/und Kromycin

Fraction II (Figure 140) was subjected to Sephadex LH-20 and then rechromatographed on a C18 column using MeOH:H_2O water to afford α-hydroxyacetovanillone (**101**) along with an inseparable mixture of kromycin (**107**) and 10,11-dihydrokromycin (**106**). The mixture was isolated as a white UV absorbing solid, which stained to yellow-brown with anisaldehyde/sulphuric acid. The mass spectrum obtained in

positive mode utilizing (+)-ESI indicated the presence of two molecular masses of 352 (**106**) and 350 (**107**) Dalton. The molecular formula of **106** was deduced as $C_{20}H_{32}O_5$ by high-resolution mass analysis (HRESI) representing 5 double bond equivalents which was one less than that of **107**.

The ^{1}H NMR spectrum of 10,11-dihydro-kromycin (**106**) displayed a methine doublet of doublet at δ 6.34 (H-5) as a result of the allyl coupling of the methyl CH_3-6 and the vicinal proton at δ 2.74 (H-6). In addition an oxygenated methine signal at δ 4.80 (dd, H-13) and a methine flanked by two carbonyls at δ 4.30 (q, H-2) was observed. Also, the spectrum exhibited four methylene multiplets of H-10 (δ 2.50, 2.36), H-11 (δ 1.83, 1.60), H-7 (1.52, 1.40) and H-14 (δ 1.71, 1.52). Finally six methyl groups at δ 1.90 (d, CH_3-4) connected to an sp^2 carbon, 1.41 (d, CH_3-2), 1.19 (s, CH_3-12), 1.04 (d, CH_3-6,8) and 0.93 (t, CH_3-15) were seen.

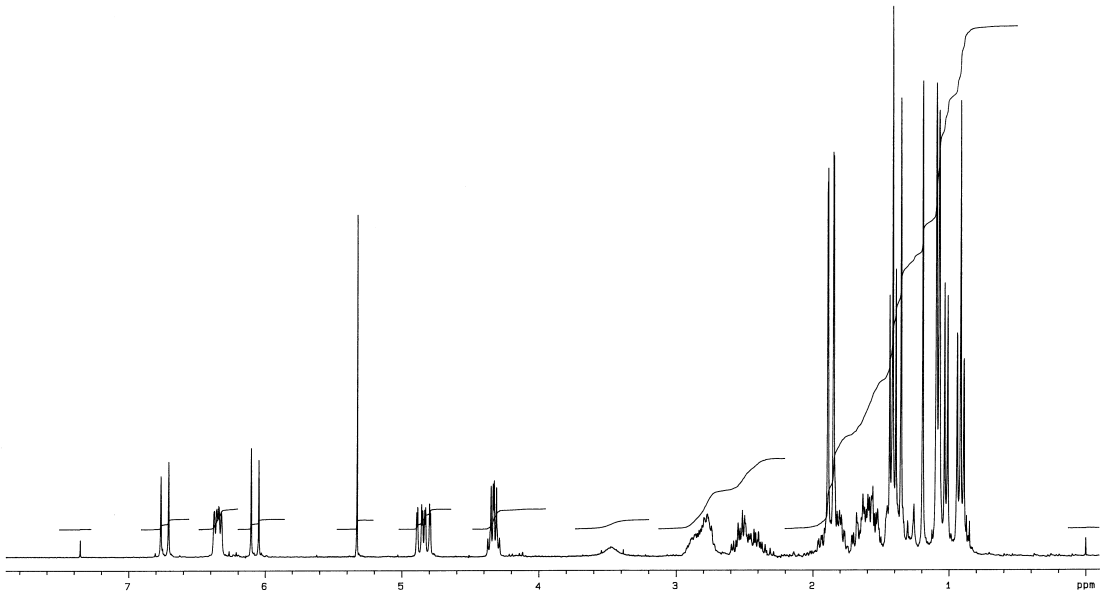


Figure 146. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 10,11-dihydro-kromycin (**106**)/ kromycin (**107**) mixture.

The ^{13}C NMR spectrum indicated the presence of three carbonyl signals at δ 215.4 (C-9), 196.1 (C-3) and 172.1, two sp^2 carbons at δ 147.4 (C-5) and137.9 (C-4), two oxygenated carbons at δ 82.3 (C-13) and 73.6 (C-12), three methine at δ 45.6, 42.0, 32.6 attributed to H-2,8,6 respectively. Finally six methyl between δ 23.4 -10.9 were seen.

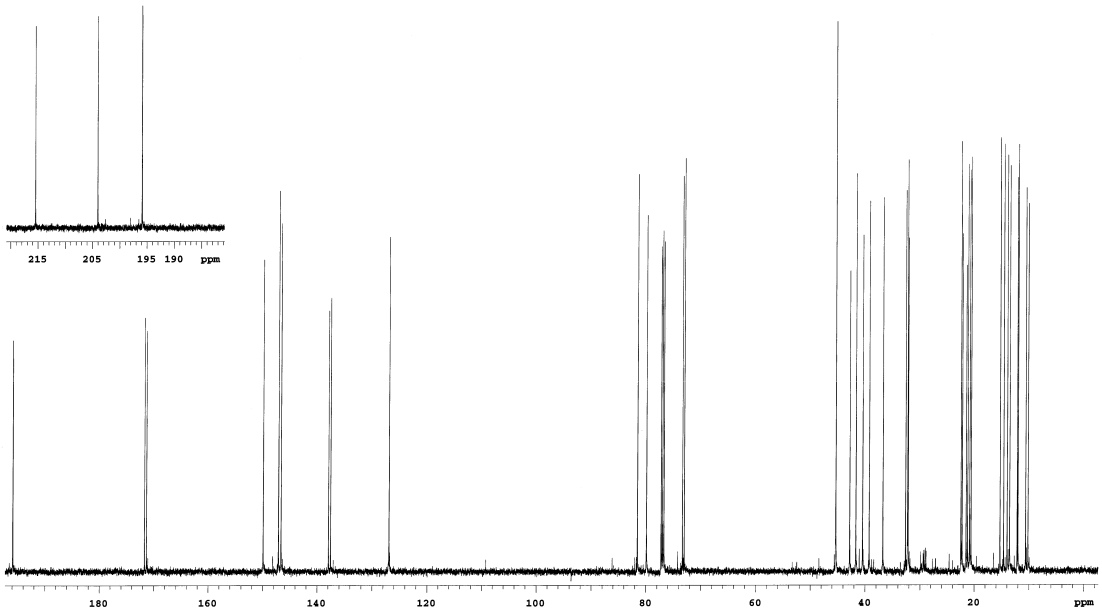


Figure 147. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 10,11-dihydro-kromycin (**106**)/ kromycin (**107**).

Searching with the above spectroscopic data in AntiBase and the Chemical Abstracts led to kromycin (**107**) for the molecular mass of 350. No hit was found for the mass of 352 Dalton, pointing to a new natural metabolite. To confirm the structure of **107** and to elucidate that of **106** the sample was submitted to 2D NMR experiments. From the H,H COSY spectrum, the following sub-structures (A-D) were established.

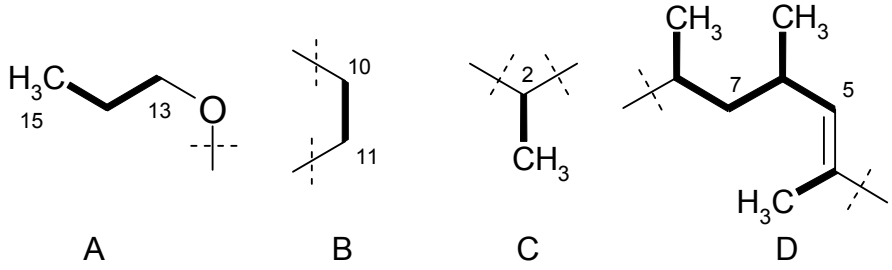


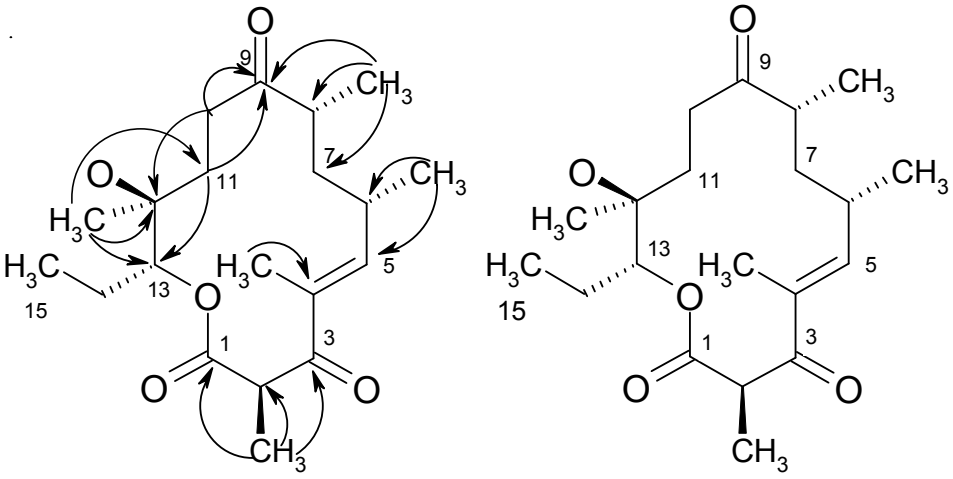
Figure 148. Fragments resulted from ^{1}H NMR and H,H COSY spectra of 10,11-dihydro-kromycin (**106**)/ kromycin (**107**) mixture.

Analysis of the HMBC spectrum showed a correlation from CH_3-8 (δ 1.04), the methine CH-8 (δ 2.46) and the three methylenes CH_2-10 (δ 2.50, 2.36), CH_2-11 (δ 1.83, 1.60), CH_2-7 (δ 1.52, 1.40) to the carbonyl C-9 (δ 215.4), which supported the connection of fragment B and D via C-9. HMBC correlations from the methyl at δ 1.19 (CH_3-12) to the two oxygenated carbons at δ 73.6 (C_q-12), and 82.3 (C-13) and the methylene carbon at δ 32.0 (C-11) located the methyl at C-12 and connected A with B via C-12 /C-13. In addition, two- and three-bond HMBC correlations from the

methyls at δ 1.90 (CH_3-4) and 1.41 (CH_3-2), the methine at δ 4.30 (H-2), the olefinic at δ 6.34 (H-5) to the carbonyls at δ 196.1 and 172.1 connected fragments C with D and located the ester CO at C-2. The molecular formula, the ^{13}C and ^{1}H NMR spectra and the number of five double bond equivalents suggested that this compound has one ring, which resulted in **106**.

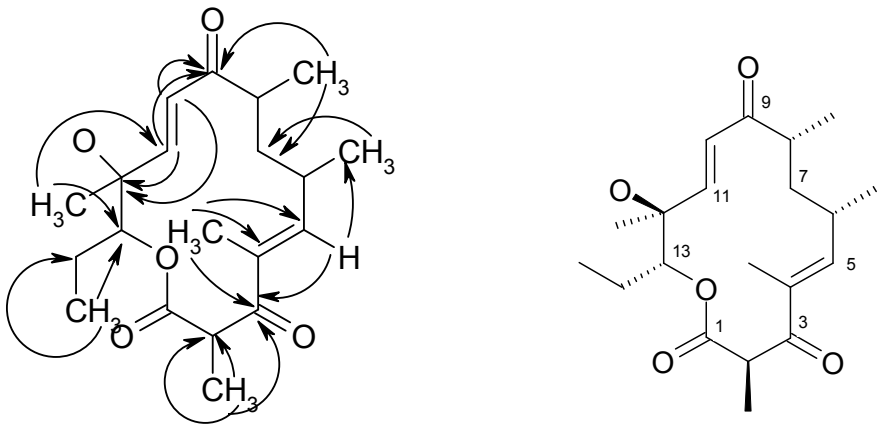
Table 14. ^{1}H NMR and ^{13}C NMR data of 10,11-Dihydro-kromycin (**106**)/ Kromycin (**107**).

	10,11-Dihydro-kromycin		Kromycin	
Position	^{1}H (Int., mult, *J* [Hz])	^{13}C	^{1}H (Int., mult., *J* [Hz])	^{13}C
1	-	172.1	-	171.5
2	4.30 (1H, q, 7.0)	45.6	4.30 (1H, q, 7.0)	45.6
CH_3-2	1.41 (3H, d, 6.9)	14.3	1.43 (3H, d, 6.9)	13.8
3	-	196.1	-	196.3
4	-	137.9	-	138.2
CH_3-4	1.90 (3H,d, 1.3)	12.3	1.91 (3H, d, 1.3)	12.5
5	6.34 (1H, dd, 1.2, 8.1)	147.4	6.33 (1 H,dd, 1.2, 6.3)	147.5
6	2.74 (1H, m)	32.6	2.84 (1H, m)	33.0
CH_3-6	1.04 (3H, d, 6.6)	20.9	1.04 (3H, d, 6.4)	21.2
7a	1.52 (1H, m)	40.7	1.58 (1H, m)	43.0
7b	1.40 (1H, m)	-	1.38 (1H, m)	
8	2.46 (1H, m)	42.0	2.77 (1H, m)	39.6
CH_3-8	1.04 (3H, d, 6.6)	15.7	1.06 (3H, d, 6.1)	15.0
9	-	215.4	3.98 (1H, m)	204.1
10a	2.50 (1H, m)	37.0	6.05 (1H, d, 16.6)	127.4
10b	2.36 (1H, m)	-		
11a	1.83 (1H, m)	32.0	6.72 (1H, d,16.6)	149.6
11b	1.60 (1H, m)	-		
12	-	73.6	-	74.0
CH_3-12	1.19 (3H, s)	23.4	1.35 (3H, s)	21.7
13	4.80 (1H, dd, 2.4, 0.8)	82.3	4.86 (1H, dd, 2.4, 10.9)	80.7
14a	1.71 (1H, m)	22.7	1.84 (1H, m)	22.1
14b	1.52 (1H, m)	-	1.50 (1H, m)	
15	0.93 (3H, t, 7.3)	10.9	0.93 (3H, t, 7.4)	10.5



106

The ^{1}H NMR sub-spectrum of kromycin (**107**) showed three olefinic proton signals at δ 6.72 (d, H-11), 6.05 (d, H-10) and 6.33 (dd, H-5) belonging to two α,β-unsaturated carbonyls, which was also confirmed by the HSQC and ^{13}C NMR spectra. Long-range correlations of H-10 and H-11 with the carbonyl CO-9 (204.1) and C_q-12 (74.0) C-7, and 7-OH with C-8, in the HMBC spectrum confirmed the structure of **107**. Kromycin is a known macrolide antibiotics and was isolated from actinomycetes. [185-189] Due to the high activity of kromycin (**107**) it was synthesized by Boeckman *et al.* in 1989.[190]



107

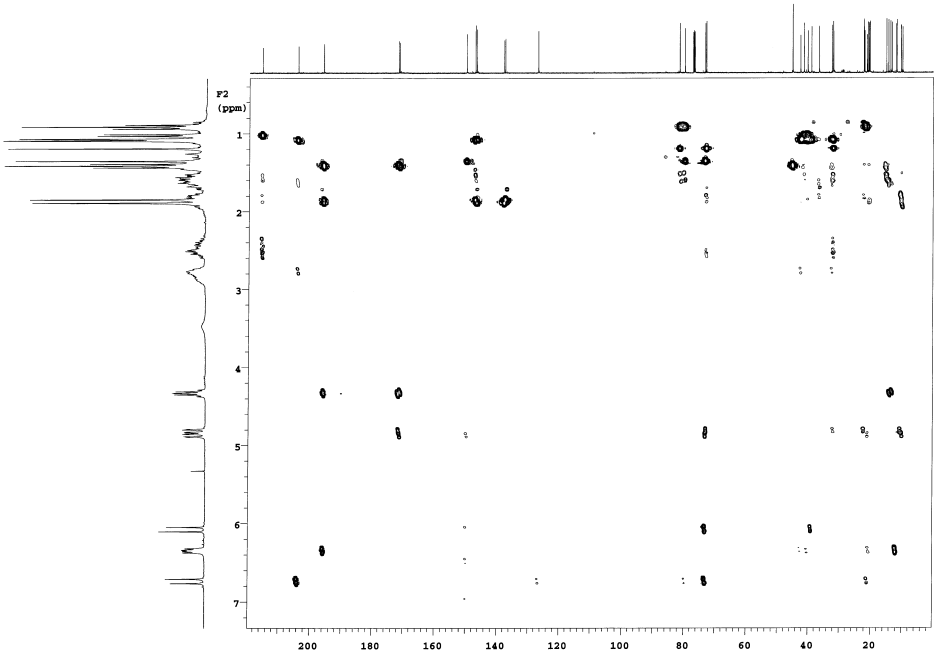


Figure 149. HMBC spectum (300 MHz, $CDCl_3$) of 10,11-dihydro-kromycin (**106**)/ kromycin (**107**) mixture.

6.6.3 Neomethymycin and Methymycin

Sub-fraction IVb (Figure 140) was chromatographed over a C18 column and eluted with methanol/water to afford a mixture of neomethymycin (**108**) and methymycin (**109**) as brown oil, which gave a pink colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed similarity to 10,11-dihydro-kromycin (**106**) and kromycin (**107**) pointing to a related structure. The ^{1}H NMR spectrum displayed overlapping signals of oxygenated carbons between δ 3.90-3.35 as well as the signal of an anomeric proton at δ 4.32, indicating a sugar moiety (desosamine sugar). (+)-HRESIMS exhibited a molecular ion at *m/z* 470.31124 $[M + H]^+$, corresponding to the molecular formula $C_{25}H_{43}NO_7$. Searching with these spectroscopic data in AntiBase and the Chemical Abstracts led to neomethymycin (**108**) and methymycin (**109**) as two possible structures.

The ^{1}H NMR spectrum of the major component neomethymycin (**108**) displayed two doublets of doublets of olefinic protons at δ 6.74 and 6.42, for which the coupling constants suggested a *trans*-orientation. The ^{13}C NMR spectrum showed two carbonyl signals at δ 204.9 (CO-7) and 174.5 (CO-1) due to a ketone and an ester, as well as to two sp^2 carbons belonging to an α,β-unsaturated carbonyl system at 147.3 (C-9) and 126.1 C-8).

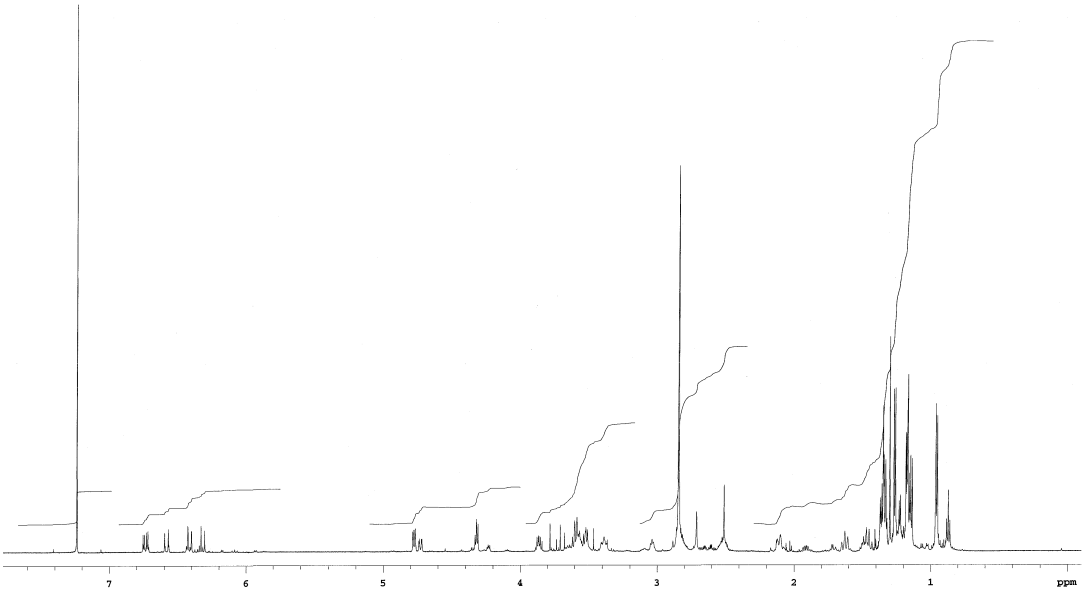
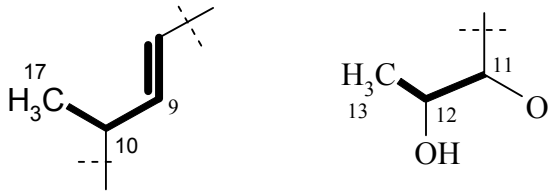


Figure 150. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of neomethymycin (**108**)/methymycin (**109**).

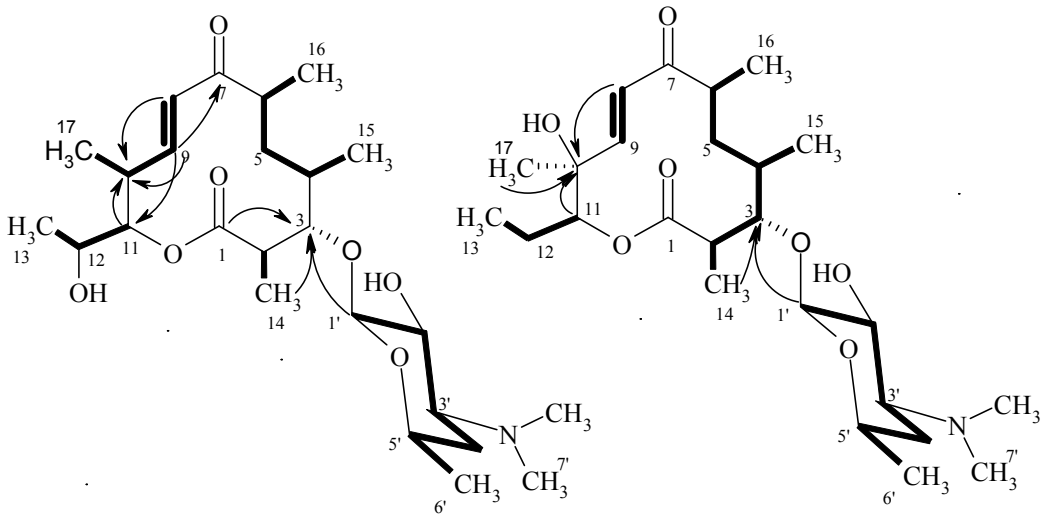
The structure of neomethymycin (**108**) was confirmed via 2D NMR experiments. In the homonuclear H-H COSY spectrum, the olefinic proton signal at δ 6.74 (H-9) was coupled with the methine at δ 3.04 (m, H-10), which was further coupled with the methyl signal at δ 1.14 (d, H-17) to establish the fragment A shown in Figure 151. A further sequence of resonances starting from the oxy-methine signal at δ 4.78 (dd, H-11) to the methine at δ 3.86 (m, 1H, H-12) and the methyl at δ 1.18 (d, ^{3}J = 7.0 Hz, 3H, H-13) was observed to afford fragment B. Analysis of the HSQC and HMBC spectra confirmed these assignments which showed correlations from H-8, H-9, H-17, H-11 to C-10 and connected fragments A and B via C-10/C-11. Finally the correlation from the anomeric proton at δ 4.32 (d, H-1') to the oxy-methine carbon C-3 (δ 85.8) located the sugar moiety at C-3.

In methymycin (**109**), the olefinic protons H-8 and H-9 appeared as two doublets indicating no protons associated with C-10, which was also supported by the fact that the methyl group at δ 1.35 (H-17) resonated as a singlet. Additionally, evidence for the structural determination of compound **109** was provided from the HMBC spectrum. Thus, the olefinic proton signal at δ 6.32 (H-8), the methyl singlet at δ 1.35 (H-17) and the oxy-methine at 4.73 (H-11) displayed correlations to the oxygenated quaternary carbon at δ 74.4 (C_q-10).



A **B**

Figure 151. Some fragments resulting from H,H COSY spectrum of neomethymycin (**108**) and methymycin(**109**).



108 **109**

Figure 152. Significant correlations observed in the H,H COSY (▬) and HMBC (→) spectra of neomethymycin (**108**) and methymycin(**109**).

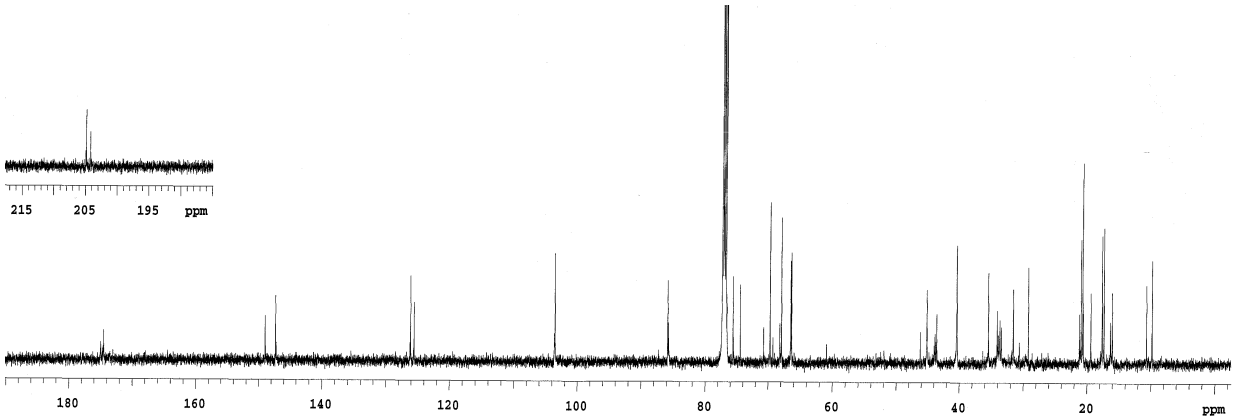


Figure 153. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of a neomethymycin (**108**)/ methymycin (**109**) mixture.

6.6.4 Picromycin

Picromycin (**110c**) was isolated from the same fraction as neomethymycin (**108**) and methymycin (**109**) using the same methods. Compound **110c** showed the same pink colour reaction with anisaldehyde/sulphuric acid and heating as **108** and **109**. The (+)-ESI mass spectrum indicated a molecular mass of 525 Dalton. Its molecular formula was established as $C_{28}H_{48}NO_8$ by high-resolution mass analysis (HRESI) of the $[M + H]^+$ ion. The ^1H NMR spectrum of picromycin (**110c**) revealed a high similarity to that of neomethymycin (**108**)/methymycin (**109**). As in the case of neomethymycin (**108**), the olefinic protons were observed at δ 6.63 (H-11), 6.31 (H-10) as two doublets, excluding vicinal protons at C-12; the anomeric proton of the desosamine sugar was seen at δ 4.35 (H-1').

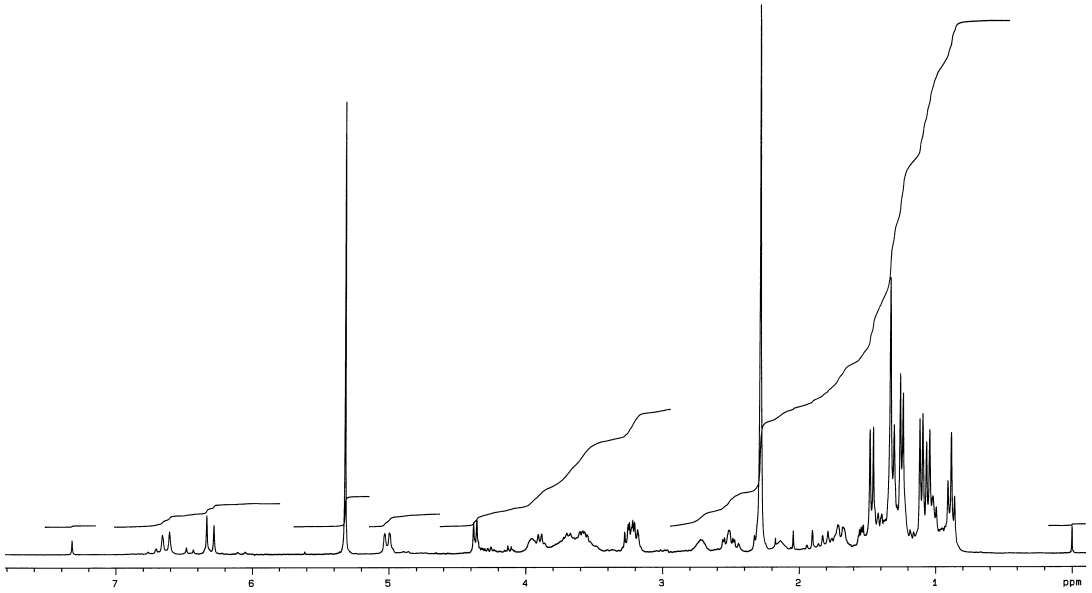
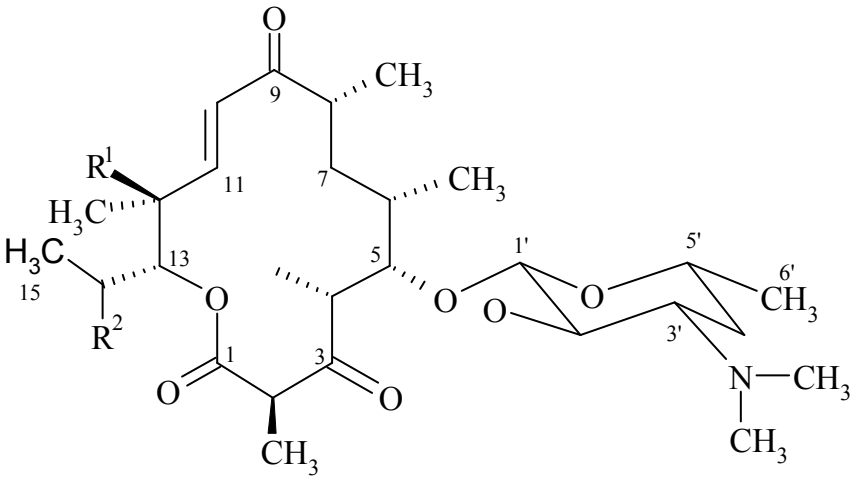


Figure 154. ^1H NMR spectrum (300 MHz, $CDCl_3$) of picromycin (**110c**).

The ^{13}C NMR spectrum revealed three carbonyl signals instead of two in **108** and **109** at δ 212.5, 203.7, 170.2, which were assigned to CO-3, CO-9 and CO-1, respectively. In addition, the signals at 145.4 (CH-11), 128.8 (CH-10), 104.8 (CH-1') were assigned to two olefinic and an anomeric carbon signal, respectively. A search in AntiBase led to three known structures: picromycin (**110c**), 5-O-mycaminosylnarbonolide (**110a**) and neopicromycin (**110b**). To continue the structure elucidation of **110c**, 2D NMR experiments were performed. The 2D NMR spectra allowed the assignment of all ^1H and ^{13}C NMR signals of the sugar moiety. On the basis of the ^{13}C-^1H correlations, the olefinic protons at δ 6.63 (d, H-11) and 6.31 (d, H-10) correlated with the oxy-methine at δ 5.01 (d, H-13) and the methyl singlet at δ 1.23 (CH_3-12) gave a cross

signal with the quaternary oxygenated carbon at δ 74.8 (C_q-12); this confirmed the structure of picromycin (**110c**).



110a R^1 = H, R^2 = H **110b** R^1 = H, R^2 = OH **110c** R^1 = OH, R^2 = H

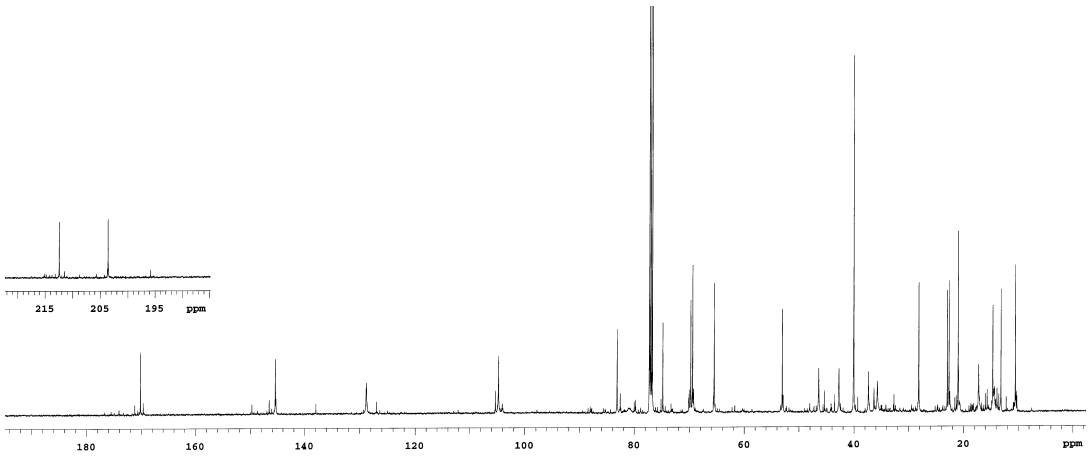


Figure 155. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of picromycin (**110c**).

Macrolide antibiotics form a large and structurally diverse class of natural products, which possess a wide range of biological activities; this makes them useful in human and veterinary medicine, agriculture, and animal nutrition.[191,192] Based on the size of the macrocyclic lactone ring of the aglycone, they were classified as 12-, 14, or 16-membered ring macrolides. Neomethymycin (**108**) and methymycin (**109**) were isolated from *Streptomyces venezuelae* ATCC 15439,[193,194] and *Streptomyces* sp. M-2140. Methymycin (**109**) is the first macrolide for which a structure was determined.[195-197] Brockmann and Henkel isolated the first macrolide antibiotic from *Streptomyces* in 1950, which they named pikromycine due to its bitter taste.[198] The activity of the producing *Streptomyces* sp. Ank22 against *Bacillus subtilis* is due to these compounds.[199, 194]

6.7 Terrestrial *Streptomyces* sp. Ank123

The crude extract of the terrestrial *Streptomyces* sp. Ank123 exhibited a good biological activity against *Streptomyces viridochromogenes* (Tü57) and weak activity against *Escherichia coli* and *Bacillus subtilis.* The chemical screening revealed several UV absorbing bands which turned to yellow, red and orange with anisaldehyde/sulphuric acid as well as to two low polar and non UV absorbing band which turned dark reddish-brown and pale yellow with the spray reagent.

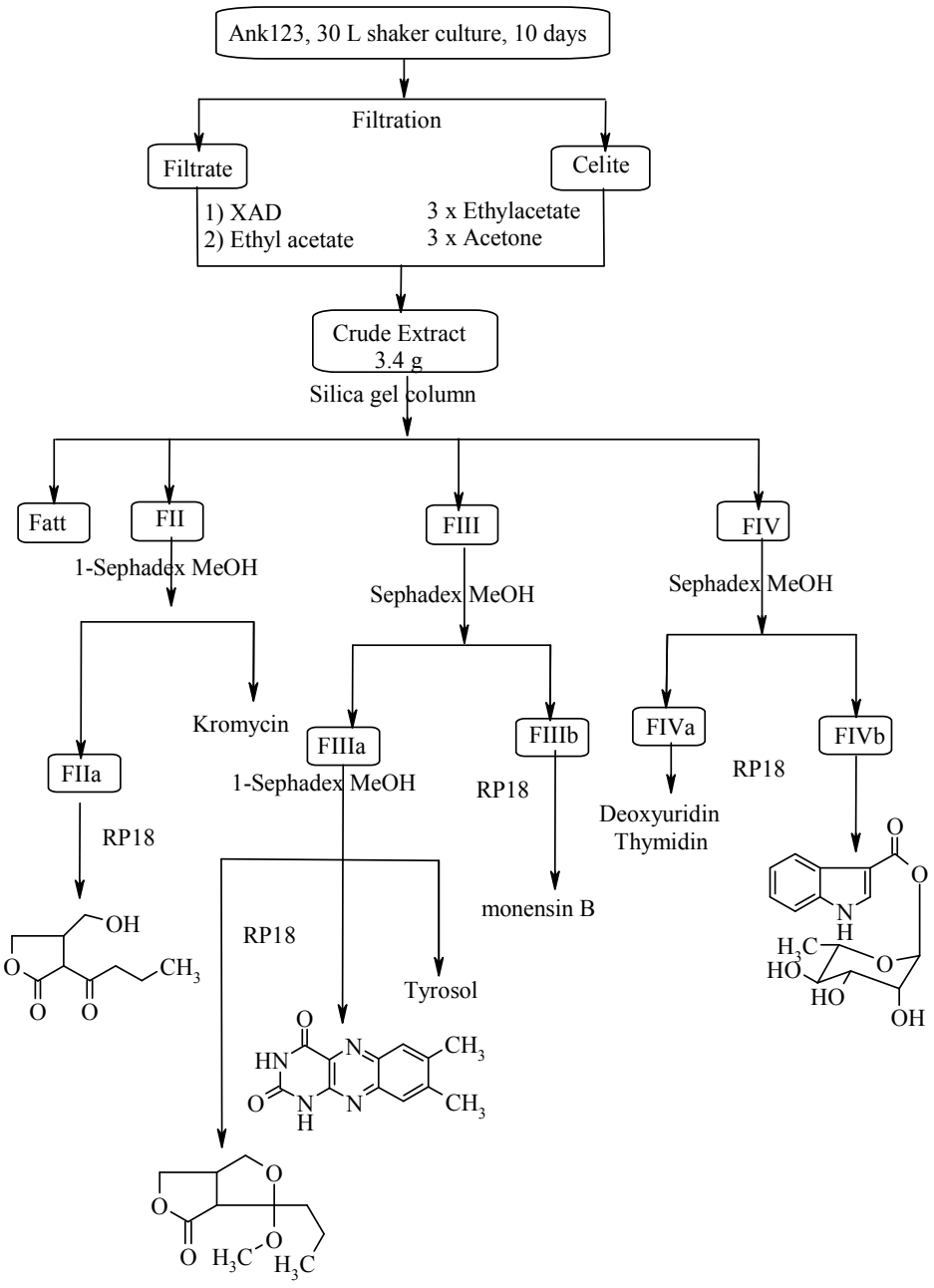
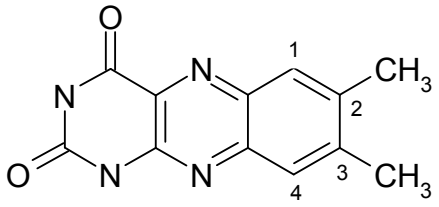


Figure 156. Work up scheme of the terrestrial *Streptomyces* sp. Ank123.

6.7.1 Lumichrome

Lumichrome was isolated from sub-fraction III (Figure 156) using Sephadex LH-20 column eluting with methanol followed by C18 column as colourless oil, which was UV absorbing at 254 nm and stained to yellow with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of lumichrome (**111**) exhibited four singlets, two in the aromatic region at δ 7.95 and 7.74, and in the aliphatic region at δ 2.53 and 2.50 attributed to two methyl groups possibly connected with an aromatic ring or nitrogen atoms, respectively. The molecular weight of 242 Dalton was deduced from the EI mass spectrum, indicating a molecular formula of $C_{12}H_{10}N_4O_2$. A search in AntiBase resulted in lumichrome (**111**), which was previously isolated from a Chinese marine sponge *Cinachyrella australiensis* by Liya *et al*. in 2004,[200] from the actinomycete *Micromonospora* sp. strain Tü 6368[201] and the fungus *Aspergillus oniki* 1784.[202] It was reported to act as a testosterone 5α-reductase inhibitor.[203]



111

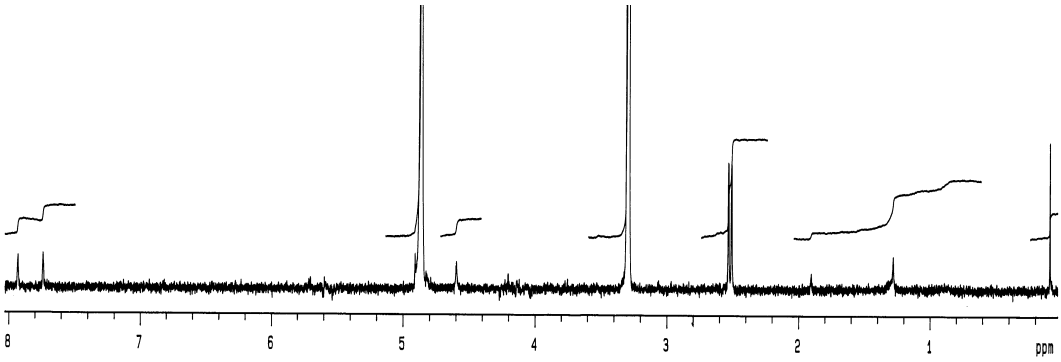
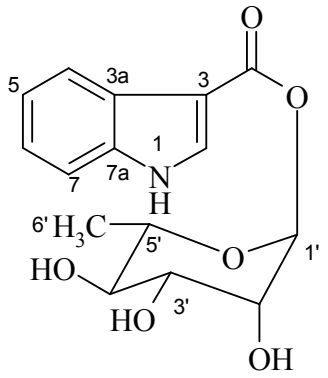


Figure 157. ^{1}H NMR spectrum (300 MHz, CD_3OD) of lumichrome (**111**).

6.7.2 Indole-3-carbonyl-L-rhamnopyranoside

TLC of fraction IV (Figure 156) showed a UV absorbing and polar band, which gave an orange colour with anisaldehyde/sulphuric acid and pink with Ehrlich's reagent, respectively, indicating the presence of an indole derivative. Fraction IV (Figure 156) was chromatographed over Sephadex LH-20 using methanol as eluting solvent followed by PR18 to afford compound **112**.

The ^{1}H NMR spectrum exhibited four aromatic proton signals at δ 8.04, 7.46, 7.22, 7.20 due to a 1,2-disubstituted benzene ring and a singlet at δ 7.21, which indicated the indole moiety. In addition, a doublet at δ 6.21 of an anomeric carbon, four methine double doublets bearing oxygen at δ 3.97, 3.91, 3.81, and 3.51 as well as a methyl signal at δ 1.28 (d, H-6') were observed, which established a pentose moiety. The ESI mass spectra determined its molecular weight as 307 Dalton, due to the presence of two *quasi*-molecular ion peaks at *m/z* 637 $[2\ M + Na]^+$ and 330 $[M + Na]^+$ in (+)-ESI mode as well as two signals at *m/z* 613 $[2\ M - H]^-$ and 306 $[M - H]^-$ in the (-)-mode. The molecular formula was deduced by HRESI mass spectrum to be $C_{15}H_{17}NO_6$. A search with this information in AntiBase resulted to indole-3-carbonyl-L-rhamnopyranoside (**112**), which was further confirmed by comparison with the data reported in the literature. Compound **112** was previously isolated from *Streptomyces* sp. GT 61150.[204]



112

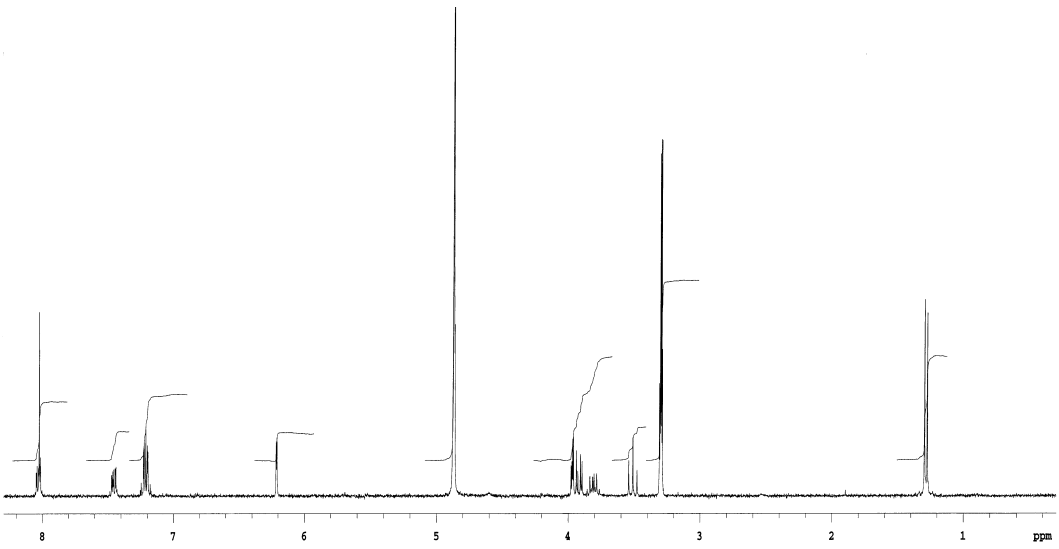


Figure 158. ^{1}H NMR spectrum (300 MHz, CD_3OD) of Indole-3-carbonyl-L-rhamnopy-ranoside (**112**).

6.7.3 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one

Compound **113** was isolated as colourless oil, which revealed no UV absorption and gave a violet colour after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **113** showed three multiplets due to two oxymethylene groups at δ 4.44, 4.14 (H-5) and 3.65 (H-10), which overlapped with a methine signal. Furthermore, the spectrum showed a methine multiplet at δ 3.20 (H-4), two methylenes at δ 2.90 (Ha-7), 2.60 (Hb-7), 1.60 (H-8) as well as one methyl doublet at δ 0.92. Compound **113** exhibited a *pseudo*-molecular ion peak in the CI mass spectrum at *m*/*z* 204 $[M + NH_4]^+$, establishing the molecular formula $C_9H_{14}O_4$.

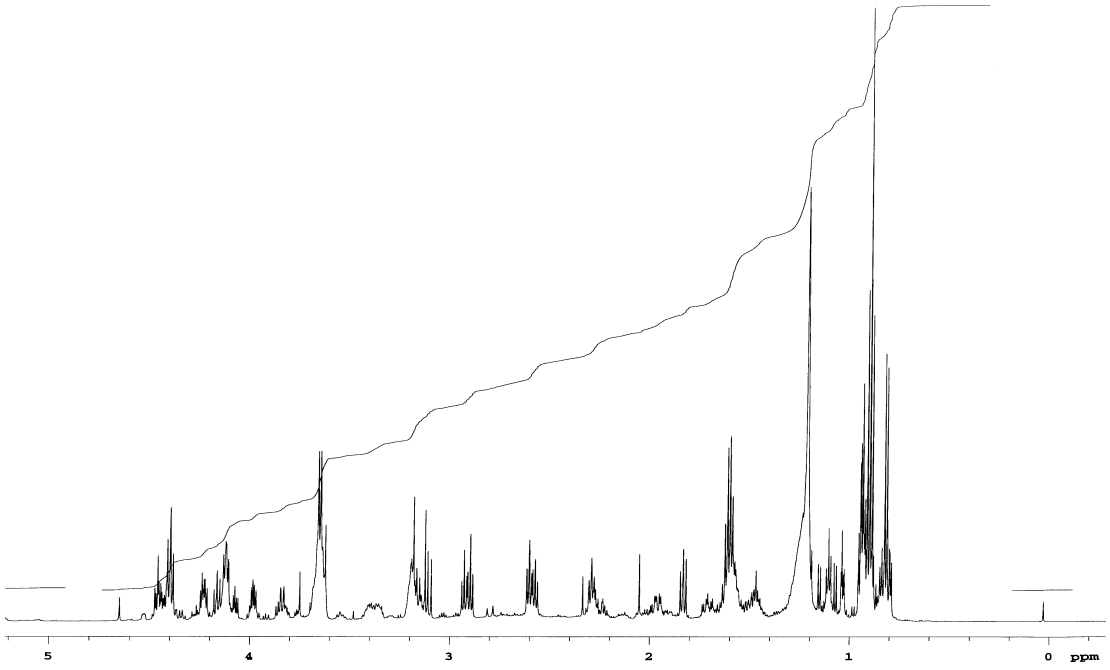


Figure 159. ^{1}H NMR spectrum (300 MHz, CD_3OD) of 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**).

The ^{13}C and HMQC NMR spectra showed nine carbon resonances, two carbonyls at δ 202.9 and 172.5 attributed to a ketone or aldehyde and an ester, respectively, two signals at δ 69.1 and 61.7 indicative for oxygenated carbons, and a methine carbon at δ 54.5 connected to a hetero atom or placed between two sp^2 carbons. Of the remaining resonances, three signals were attributed to two methylenes at δ 44.3 connected to sp^2 carbon and 16.7 as well as to methyl carbons at δ 13.4. A search in AntiBase and the Chemical Abstracts resulted in compound **113** as a possible structure. Based on the H,H COSY spectrum two fragments A and B could be obtained (Figure 161).

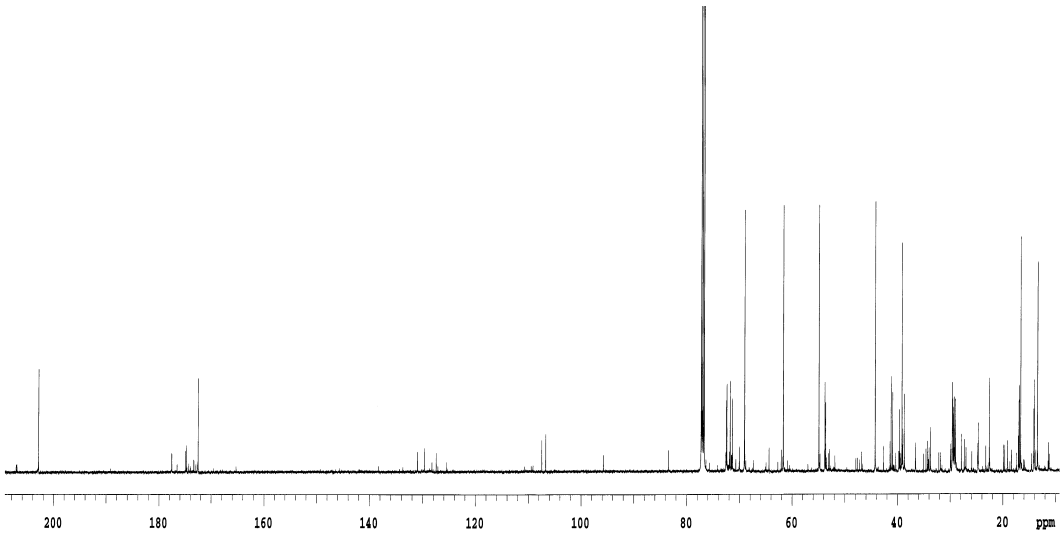


Figure 160. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**).

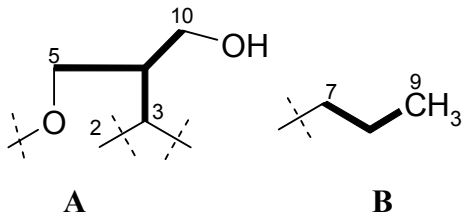
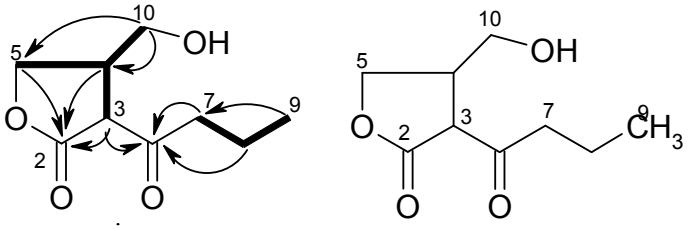


Figure 161. Fragments A and B resulted from the H,H COSY spectrum of 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**).

On the basis of the above findings and other correlations in the HMBC spectra, the two fragments shown above were confirmed. In addition, the HMBC exhibited three bond correlation from the methylene (CH_2-5) at δ 4.44 and 4.14 and the methine at δ 3.20 to the carbonyl carbon at δ 172.5 which established the lactone moiety. Finally the spectrum showed two bond correlation from the methine at δ 3.65 and the methylene (CH_2-7) at δ 2.90 and 2.60 to the carbonyl carbon at δ 202.9 which connected the two fragments A and B. Structure **113** was further confirmed by comparison with the literature data.[205]



113

Figure 162. Selective HMBC (→) and H,H COSY (▬) correlation of 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**).

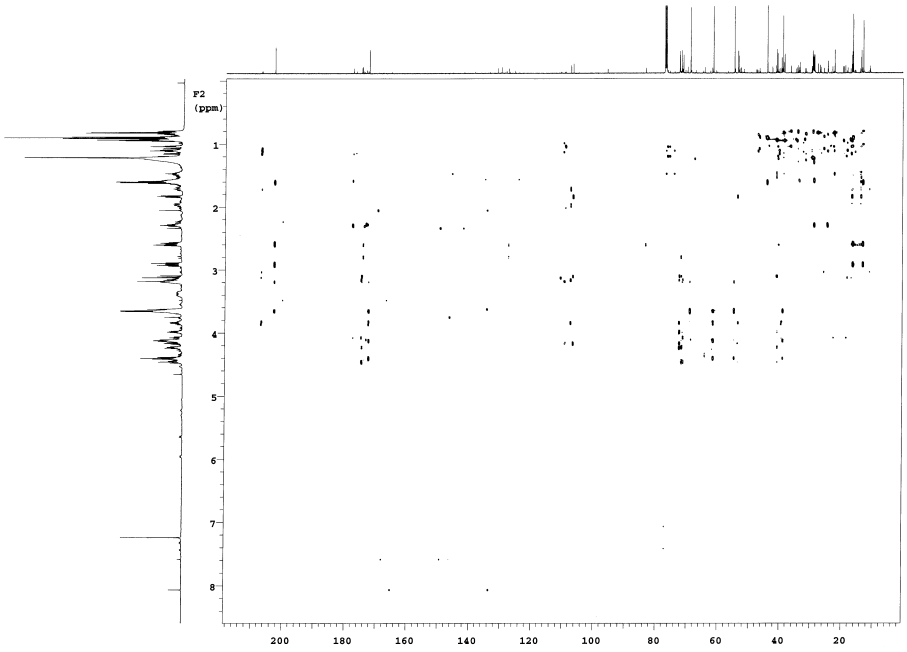
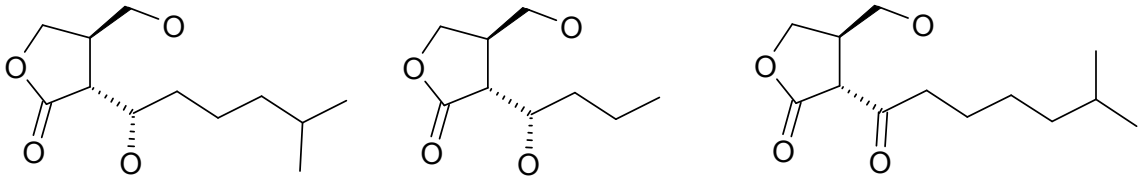


Figure 163. HMBC spectrum (600 MHz, CD_3OD) of 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**).

The butyrolactone **113** belongs to a group of autoregulators, which were isolated from the genus *Streptomyces* and are considered as microbial hormones, which control the production of secondary metabolites and are essential for the morphological differentiation such as aerial mycelium formation.[206] Based on their structural differences in the C-2 side chain, butyrolactone autoregulators can be classified into three types (i) the virginiae butanolide (VB) type, like VB-A (**114**), which have a 6-*α*-hydroxy group, controlling virginiamycin production,[207-210] (ii) the IM-2 type, such as IM-2 (**115**), which was isolated from *Streptomyces* sp. FRI-5 and has a 6-β-hydroxy group, controlling the production of a blue pigment and nucleoside antibiotics[211,212] and (iii) the A-factor (**116**) type, which possesses a 6-keto group such as the A-factor of *Streptomyces griseus*.[213-215] According to this classification, compound

113 belongs to the A-factor type; it was reported that it is one of the inducers of virginiamycin production in *Streptomyces virginiae*.[216]



VB-A (114) **IM-2 (115)** **A-factor (116)**

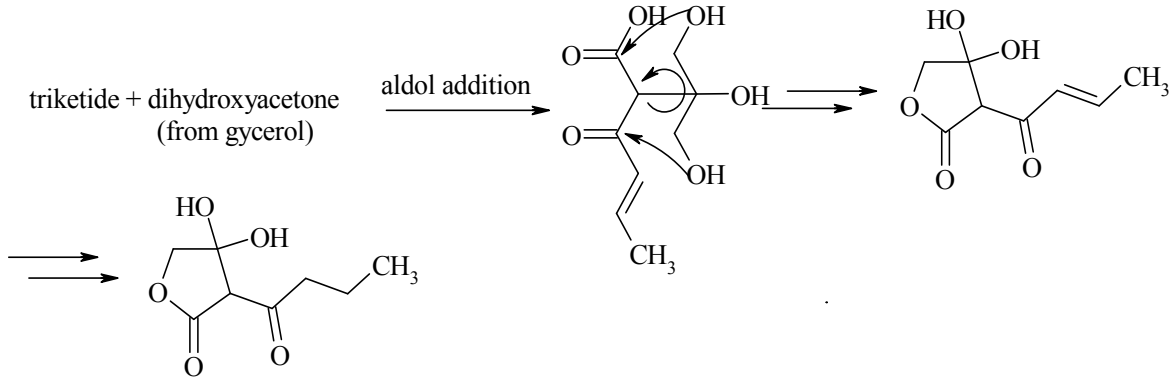


Figure 164. The mixed acetate-glycerol biosynthetic pathway to 3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**) according to Lit.[205]

6.7.4 6-Methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one

The ^{1}H NMR spectrum of the colourless oily **117** revealed close similarity to that of **113**. The main difference was a methoxy signal observed in **117** at δ 3.19. For compound **117**, a molecular formula of $C_{10}H_{16}O_4$ was obtained from the HRESI mass spectrum, which indicated a molecular mass of 14 amu and one double bond equivalent more than that of the parent compound **113**.

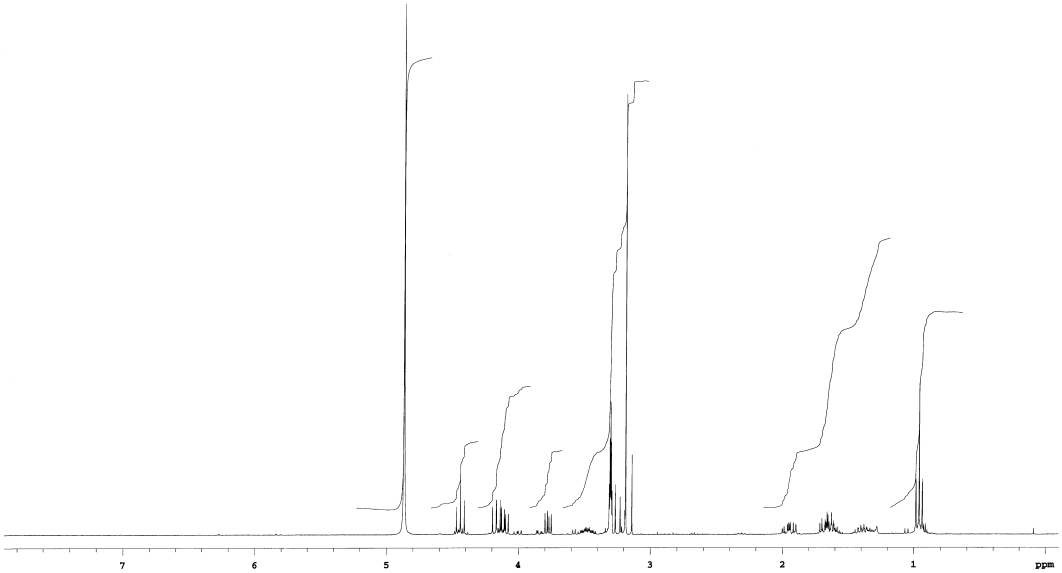


Figure 165. ^{1}H NMR spectrum (600 MHz, CD_3OD) 6-methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one (**117**).

In the ^{13}C NMR spectrum, 10 carbon signals were observed which could be classified based on the HSQC spectrum as one ester carbonyl at δ 176.9 (CO-1), one acetal carbon at δ 110.7 (C-6), two oxygenated methylenes at δ 74.1 (C-3), 73.2 (C-4), two methine connected to sp^2 carbons at δ 56.2 (C-3a), 42.8 (C-3b), two methylenes at δ 36.3 (C-7), 18.2 (C-8), one methoxy at δ 48.2 (OCH_3-6) and methyl at δ 18.2 (C-9). A search in AntiBase and the Chemical Abstracts gave no results, pointing to a new natural product from microorganisms.

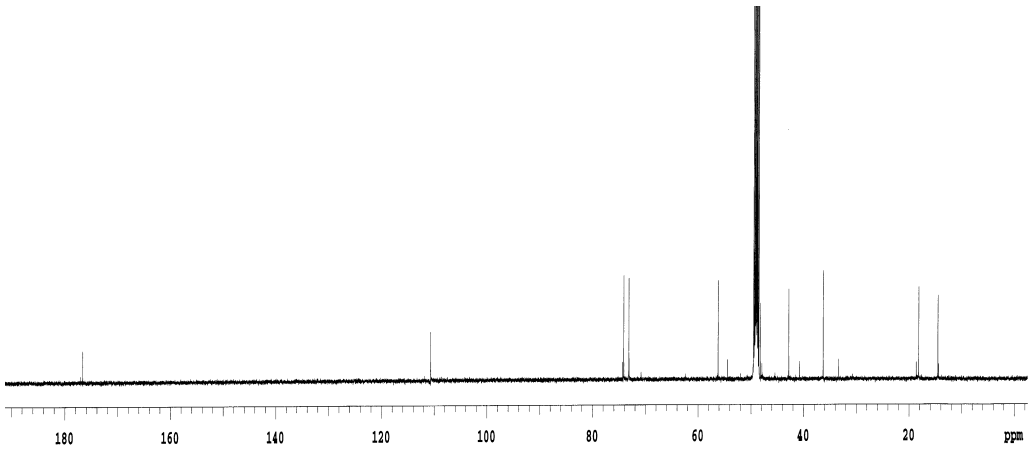
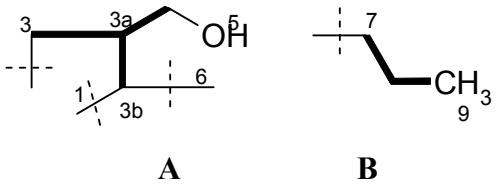


Figure 166. ^{13}C NMR spectrum (125 MHz, CD_3OD) 6-methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one (**117**).

The COSY spectrum showed ^{1}H-^{1}H correlations of a spin system including the methylenes CH_2-3 (δ 4.44, 4.10) and CH_2-4 (δ 4.17, 3.78), the sp^3 methines at δ 3.48

(H-3a) and 3.25 (H-3b) which established fragment A. Additionally, the COSY spectrum showed couplings between the methylenes CH_2-7 (δ 1.95, 1.66), CH_2-8 (δ 1.63, 1.38) and the methyl at δ 0.96 (H-9) to give fragment B.



A **B**

Figure 167. Fragments resulted from H,H COSY(—) spectrum of 6-methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one (**117**).

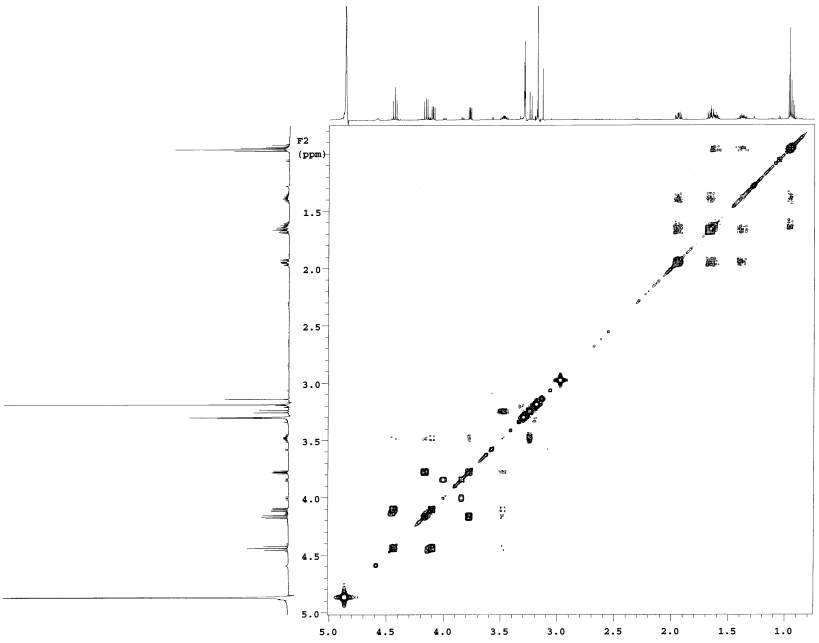
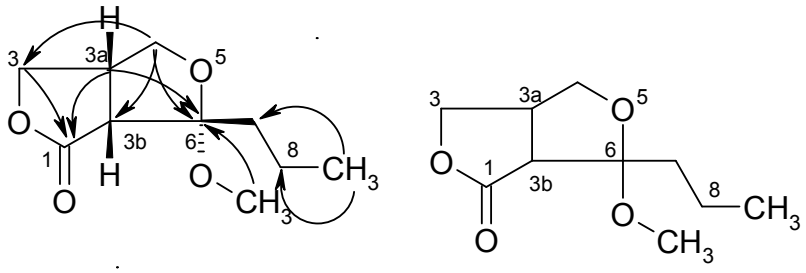


Figure 168. H,H COSY spectrum (600 MHz, CD_3OD) 6-Methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one (**117**).

Analysis of the HMBC spectrum indicated a ^3J coupling from CH_2-3 (δ 4.44, 4.10) and the methine H-3a to the carbonyl carbon CO-1 that established the lactone ring. The fused furan ring, the connection of the methoxy group and the side chain to C-6 were established by long-range couplings observed between the methine H-3a, the methylenes CH_2-4, 6, 7 and the methoxy group to the acetal carbon C-6 at δ 110.7.



117

Figure 169. HMBC correlations of (→) 6-Methoxy-6-propyl-tetrahydrofuro[3,4-c]furan-1-one (**117**).

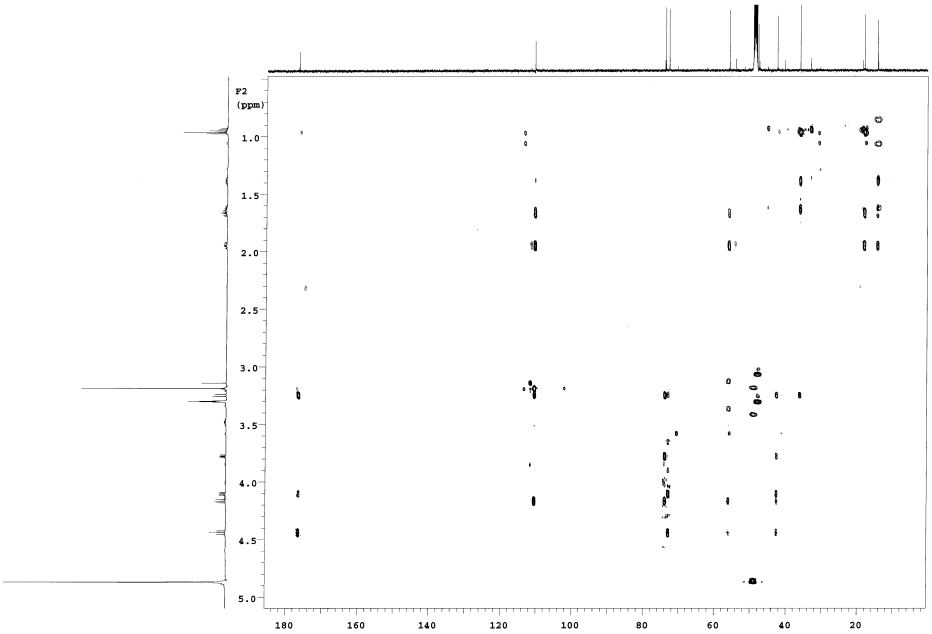


Figure 170. HMBC spectrum (600 MHz, CD_3OD) of 6-Methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one (**117**).

Based on the NOE experiment, it was clear that the two hydrogens were in *cis* position. Irradiation of the methyl of the propyl (CH_3-9) moiety resulted in a correlation with the methoxy group. However, no correlation was observed from the methyl of the propyl group to the methylenes at position 4 and 3. This suggested that the propyl group was *syn* to the hydrogens (3a and 3b) which resulted in a new secondary metabolites **117**.

6.7.5 Monensin B

TLC of sub-fraction IIIb (Figure 156) exhibited a colourless and non UV absorbing spot, which turned dark brown after spraying with anisaldehyde/sulphuric acid

and heating. Purification of this sub-fraction using C18 column eluting with methanol/water resulted in monensin B (**119c**) as brownish solid. The ESI mass spectrum of **119c** exhibited *pseudo*molecular ion peaks at 679 $[M + Na]^+$ in the positive mode. HRSIMS analysis suggested a molecular formula of $C_{35}H_{60}O_{11}$.

The ^1H NMR resonances of **119c** in methanol exhibited signals for seven methine protons on oxygenated sp^3 carbons, one oxymethylene at δ 3.92 (H-26) as well as a methoxy group at δ 3.37 (H-35). In addition overlapped signals for six methine and seven methylenes between 1.3-2.5 were observed. Finally the ^1H NMR spectrum showed signals for eight methyl groups in the range of δ 1.45-0.83.

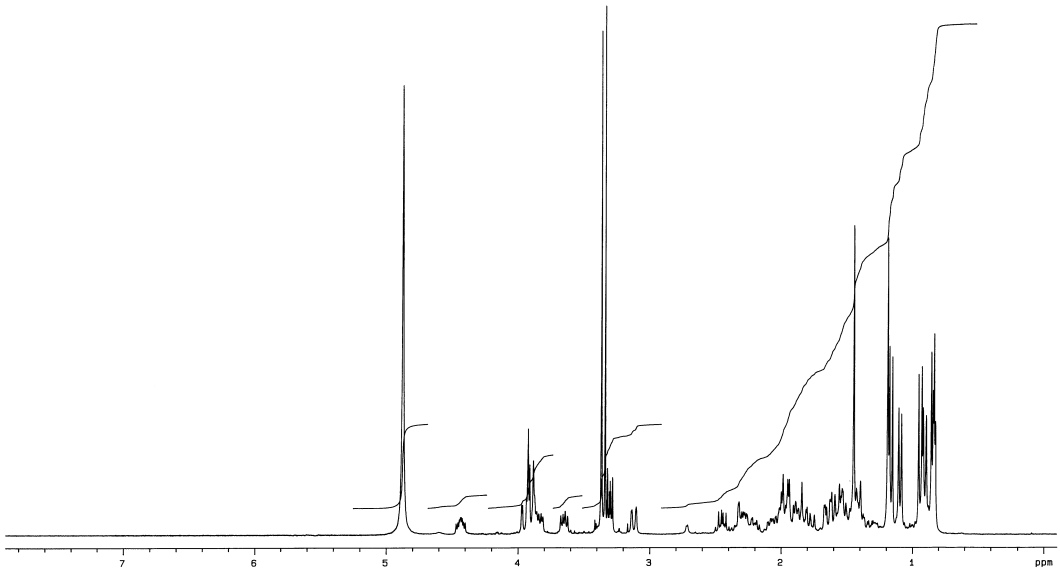
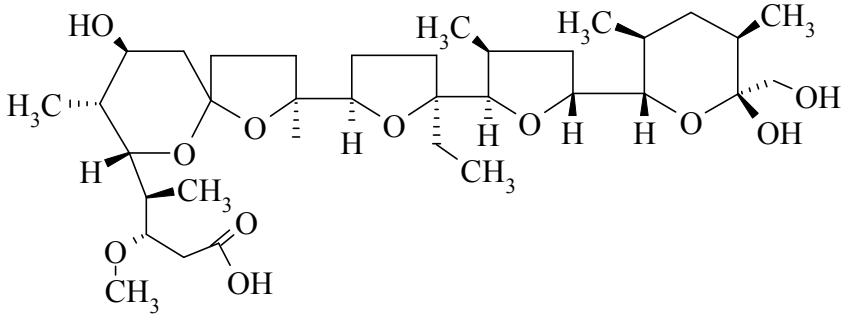


Figure 171. ^1H NMR spectrum (300 MHz, CD_3OD) of monensin (**119c**).

The ^{13}C and HSQC NMR spectra of **119c** in methanol afforded evidence of one carbonyl of carboxylic acid at δ 183.4 (C-1), thirteen signals of oxygenated sp^3 carbons including two anomeric quaternary carbons at δ 108.4 (C-9) and 99.1 (C-25), seven methines in the range of δ 88.0-69.1, another two quaternary at δ 86.8 (C_q12), 85.1 (C_q-16), one methylene at δ 65.7 (C-26) as well as one methoxy group at δ 58.3 (C-35). There were further 28 signals between δ 46 and 11, which were further analysed by HSQC spectra. Searching in AntiBase and Chemical Abstracts using the previous spectroscopic data resulted in two possible structures: monancin B (**119c**) and 2-demethylmonensin A (**118**). To elucidate the structure, it was subjected to 2D NMR experiments.



118

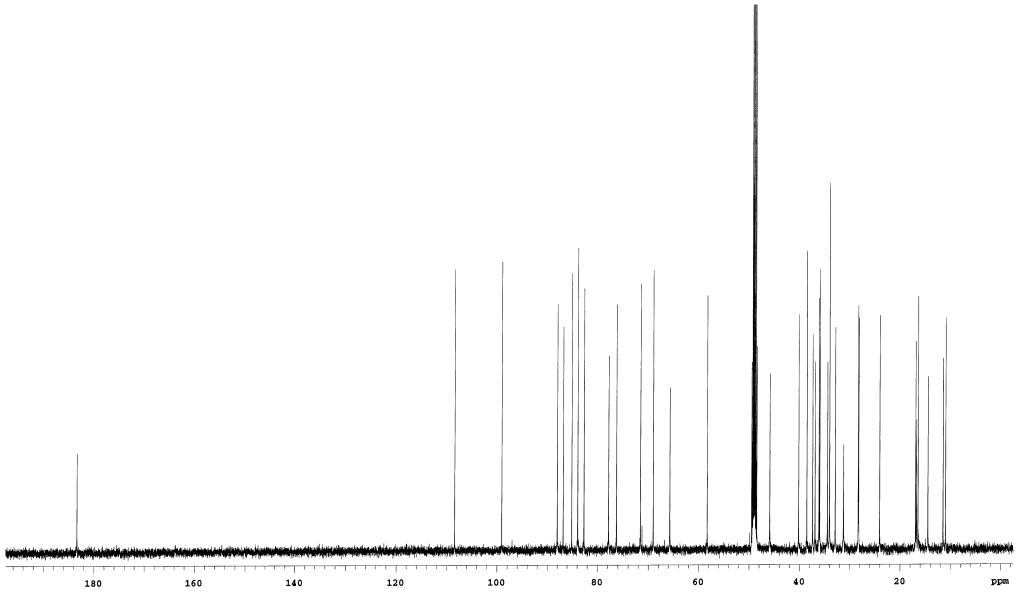
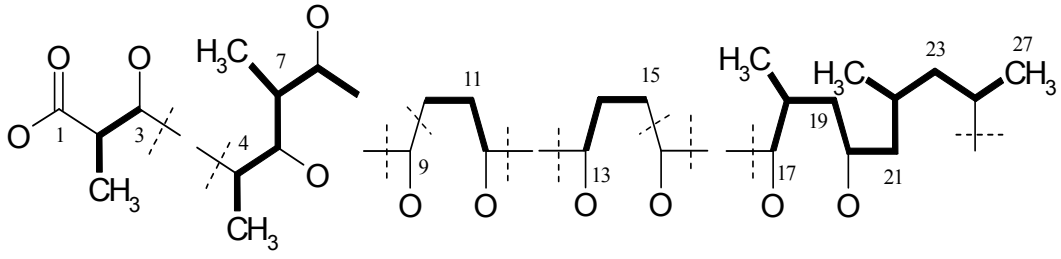


Figure 172. ^{13}C NMR spectrum (125 MHz, CD_3OD) of monensin (**119c**).

The H,H COSY spectrum showed correlation from the methine proton at δ 2.45 to the methine at δ 3.12 and the methyl group at δ 1.17 which established fragment A. The oxygenated methine at δ 4.00 correlated to another two methine protons at δ 2.06 (H-4), 2.00 (H-6) which in their turn correlated to the methyls at δ 1.10 (H-33) and 0.94 (H-32) respectively, this resulted in fragment B. The continued elucidation of the H,H COSY spectrum afforded the other fragments C, D, E (Figure 173).



A **B** **C** **D** **E**

Figure 173. Fragments resulted from H,H COSY(▬) spectrum of monensin B (**119c**).

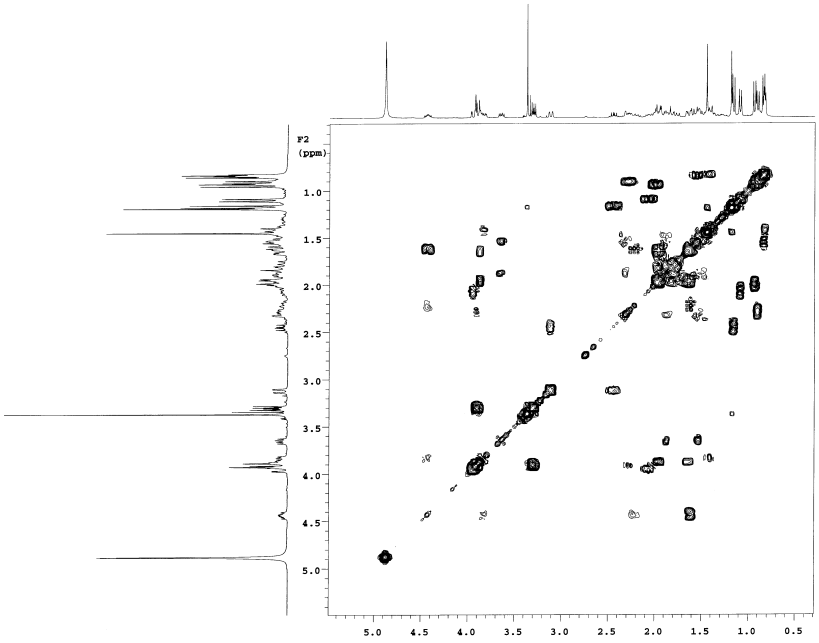


Figure 174. H,H COSY spectrum (300 MHz, CD_3OD) of monensin B (**119c**).

The HMBC spectrum exhibited three bond correlations from the methyls at δ 1.17 (H-34) and 1.10 (H-33) to the oxygenated carbon at δ 83.9 (C-3) which connected fragment A and B via C-3/C-4. The former methyl showed also correlation to the carbonyl carbon at δ 183.4 (C-1). The methylene protons at δ 1.94, 1.61 (H-8) and 1.96 (H-10) displayed ^3J coupling with the anomeric carbon at δ 108.4 (C-9) to connect fragment B with C via C-9. Furthermore, fragment C and D were connected through the methyl singlet at δ 1.45 (H-30) which showed strong coupling to the carbons at δ 86.8 (C-12), 82.7 (C-13) and 34.5 (C-11), respectively, which also excluded the 2-demethylmonensin A (**118**). Finally fragment D and E were connected via the correlation of the methyl at δ 1.19 (C-30) to the oxygenated carbons at δ 85.1 (C-16) and 88.0 (C-17) to afford monensin B (**119c**).

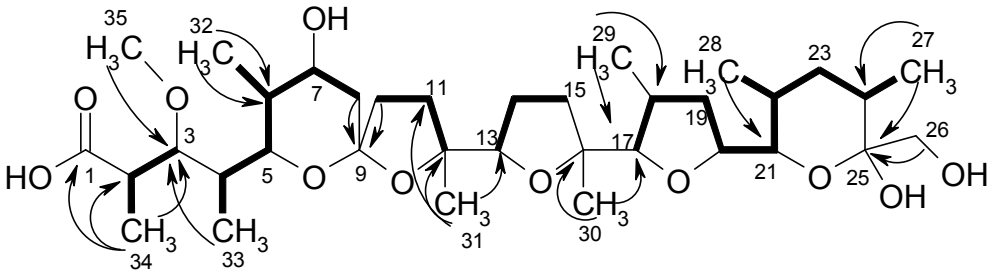


Figure 175. The H,H COSY (▬) and HMBC(→) correlation of monensin B (**119c**).

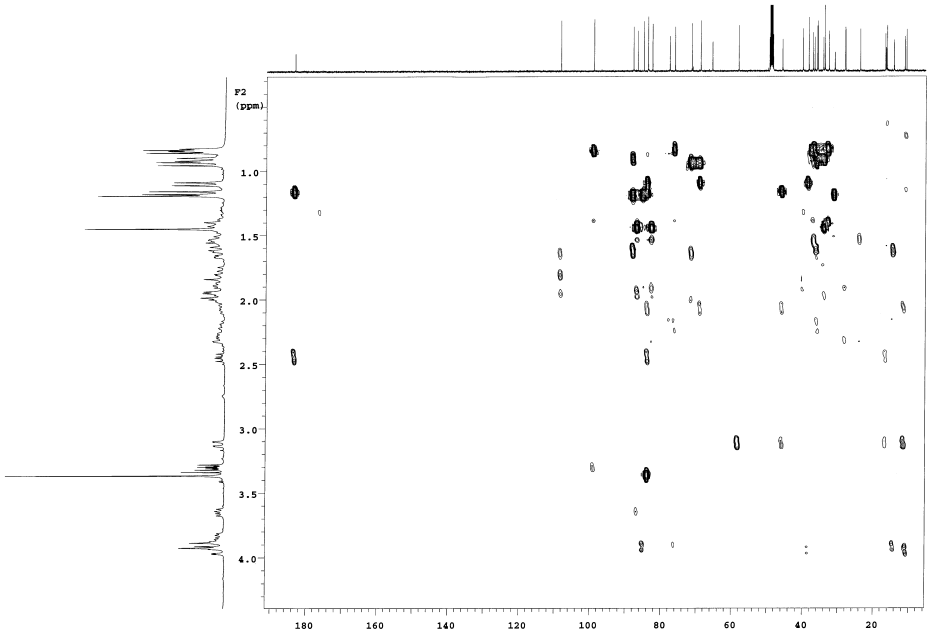


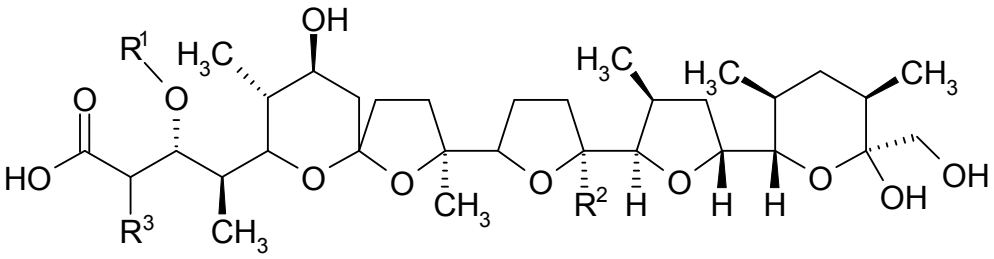
Figure 176. HMBC spectrum (300 MHz, CD_3OD) of monensin B (**119c**).

Table 15. ^{1}H NMR (300 MHz) and ^{13}C NMR (125 MHz) data of monensin B (**119c**) in CD_3OD.

Monensin B					
C#	^{1}H (Int., mult, *J* [Hz])	^{13}C	C#	^{1}H (Int., mult, *J* [Hz])	^{13}C#
1	-	183.4	19	2.32 1.61 (2H, m)	34.0
2	2.45 (1H, dd, 6.7, 10.2)	45.9	20	4.43 (1H, m, 9.6, 3.9)	77.8
3	3.12 (1H, dd, 10.2, 1.3)	83.9	21	3.84 (1H, m)	76.3
4	2.06 (1H, m)	38.6	22	1.44 (1H, m)	32.9
5	4.00 (1H, dd, 11.3, 2.1)	69.1	23	1.39 (2H, m)	36.9
6	2.00 (1H, m)	36.2	24	1.56 (1H, m)	37.4
7	3.88(1H, m)	71.6	25	-	99.1
8	1.94, 1.61 (2H, m)	34.5	26	3.92 (1H, m)	65.7
9	-	108.4	27	0.85 (3H, d, 3.9)	16.5
10	1.96 (2H, m)	40.2	28	0.83 (3H, d, 3.2)	17.0
11	1.96, 1.64 (2H, m)	34.0	29	0.91 (3H, d, 6.9)	14.5
12	-	86.8	30	1.19 (3H, s)	24.1
13	3.65 (1H, dd, 15.5, 5.2)	82.7	31	1.45 (3H, s)	28.2
14	1.88, 1.52 (2H, m)	28.3	32	0.94 (3H, d, 7.2)	11.0
15	2.32, 1.52 (2H, m)	31.2	33	1.10 (3H, d, 6.9)	11.5

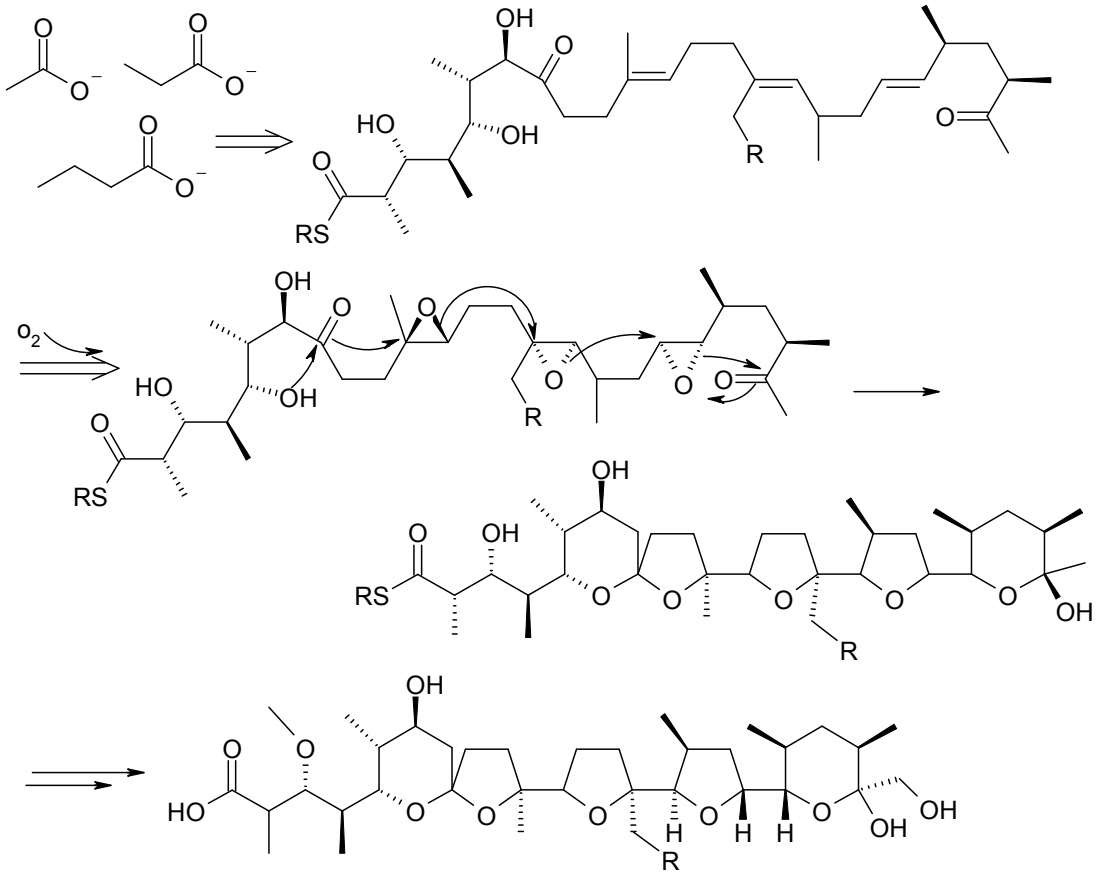
16	-	85.1	34	1.17 (3H, d, 6.7)	16.8
17	3.92 (1H, d, 3.5)	88.0	35	3.37 (3H, s)	58.3
18	2.27 (2H, m)	36.0			

Monensins are important polyether ionophore antibiotics, isolated from *Streptomyces cinnamonensis*. For example, monensin (**119a**) was broadly used as an anticoccidial agent for poultry and to improve the efficiency of feed used in ruminant animals. Monensin A (**119b**) and B (**119c**) also displayed activities against *Bacillus subtilis*,[217] which agrees with the result from the biological pre-screening. The biosynthetic pathway of monensin has attracted a great deal of interest[218,219] and it has been proposed that the cyclic ether groups in monensin might proceed via a cascade of cylisation steps on a triepoxi-intermediate which was supported by isotope labelling experiments.[220-222]



119a 119b 119c

	R^1	R^2	R^3
Monensin (**119a**)	CH_3	H	CH_3
Monensin A (**119b**)	CH_3	CH_3CH_2	CH_3
Monensin B (**119c**)	CH_3	CH_3	CH_3



Monensin A **(119b)** R = CH_3 Monensin B **(119c)** R = H

Figure 177. Biosynthetic pathway for monensin A (**119b**) and B (**119c**).

6.8 *Streptomyces diastaticus* var. 108

The polyenes macrolides constitute a group of polyketides, which is characterized by a large macrolactone ring with up to seven conjugated double bonds, forming chromophors responsible for their characteristic physical and chemical properties (strong light absorption, photolability, poor solubility in water).[223,224] The antifungal activity of these compounds is due to interactions between the polyene molecules and sterol-containing membranes. This interaction results in anion channel formation, and the membranes become permeable, causing destruction of the electrochemical gradient and subsequent cell death.[225]

As this interaction is stronger with ergosterol of fungal cells than with cholesterol of mammalian cells, a selective antifungal activity of most polyene-macrolides results, which is responsible for their high medical importance. The residual cholesterol interaction causes, however, toxic side effects during the treatment of systemic mycoses, which restrict their application to severe cases. Another disturbing factor is the

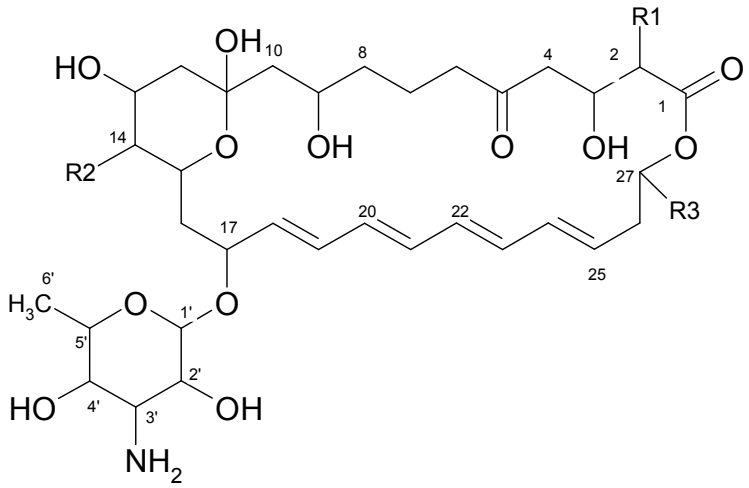
poor water solubility, which requires the use of detergents like bile acids or solvents, which are causing side reactions as well.

For these reasons, many attempts were made to synthetically modify natural polyene antibiotics, e.g. by attaching of sugar moieties to increase the water solubility. Another approach uses genetic alterations of the biosynthetic gene cluster: As the molecules are synthesized by a type I polyketide synthase, alterations in the number of modules for the elongation step should result in molecules with modified ring size.

The group of Prof. Malpartida in Madrid investigates additionally the biosynthetic conversion of the free carboxy group in polyene macrolides like rimocidin (**120f**) into the respective amides: Rimocidin B (**120g**) and CE-108B (**120b**) have been produced by genetically modified *Streptomyces diastaticus* var. 108. The genetic and biochemical analysis of the producer strain show that the two amides are derived from the parental polyenes rimocidin (**120f**) and CE-108 (**120**) by a post-PKS modification of the free side chain carboxylic acid. In this modification, amidotransferase activity generated the target compounds rimocidin B (**120g**) and CE-108B (**120b**).[226]

A further *Streptomyces* mutant was expected to produce the homologue CE-108D (**120d**) and additionally the amide CE-108E (**120e**). It was the aim of a recent cooperation with Prof. Malpartida, to isolate these compounds and to determine their structures by spectroscopic methods.

The fermentation, isolation and purification of the samples were done in Spain. The samples arrived for structure elucidation after purification by HPLC. Both macrolides were faint yellow solids. Compound CE-108D (**120d**) was sparingly soluble in methanol and good soluble in DMSO and pyridine while CE-108E (**120e**) was good soluble in methanol and DMSO.



120

		R^1	R^2	R^3
120a	CE-108	CH_2CH_3	COOH	CH_3
120b	CE-108B	CH_2CH_3	$CONH_2$	CH_3
120c	CE-108C	CH_2CH_3	CH_3	CH_3
120d	CE-108D	CH_3	COOH	CH_3
120e	CE-108E	CH_3	$CONH_2$	CH_3
120f	Rimocidin	CH_2CH_3	COOH	$CH_2CH_2CH_3$
120g	Rimocidin	CH_2CH_3	$CONH_2$	$CH_2CH_2CH_3$
120	Rimocidin	CH_2CH_3	CH_3	$CH_2CH_2CH_3$

6.8.1 CE-108D

The HRESI mass spectrum of the pale yellow powdery CE-108D (**120d**) showed a *pseudo*molecular ion peak at *m/z* 726.36927 $[M + H]^+$, which corresponds to the molecular formula $C_{36}H_{55}NO_{14}$, and fits only with structure **120d** amongst the alternatives listed above. The ^{1}H NMR spectrum showed very close similarity to those of **120a**, **b**, **c**, **f**, **g**, **h**.[226-228]

The ^{1}H NMR spectrum displayed four signals in the sp^2 region at δ 6.32 (1H, m), between δ 6.05–6.15 (5H, m), at δ 5.89 (1H, dd), and at δ 5.65 (1H, m), with integration of eight protons in total. Two exchangeable signals appeared at δ 7.18 and 5.20 as broad singlets. One oxygenated methine at δ 4.53 was due to an anomeric proton, according to the HSQC spectrum. The other protons of the sugar moiety appeared in the range of δ 4.62-3.25; a methine proton at δ 2.81 was possibly connected to an sp^2 carbon or nitrogen atom. In the aliphatic region between δ 2.60-1.40, the spectrum showed a complex multiplet pattern, in addition to three methyl doublets: two at δ 1.17 and the third at δ 1.08 were present.

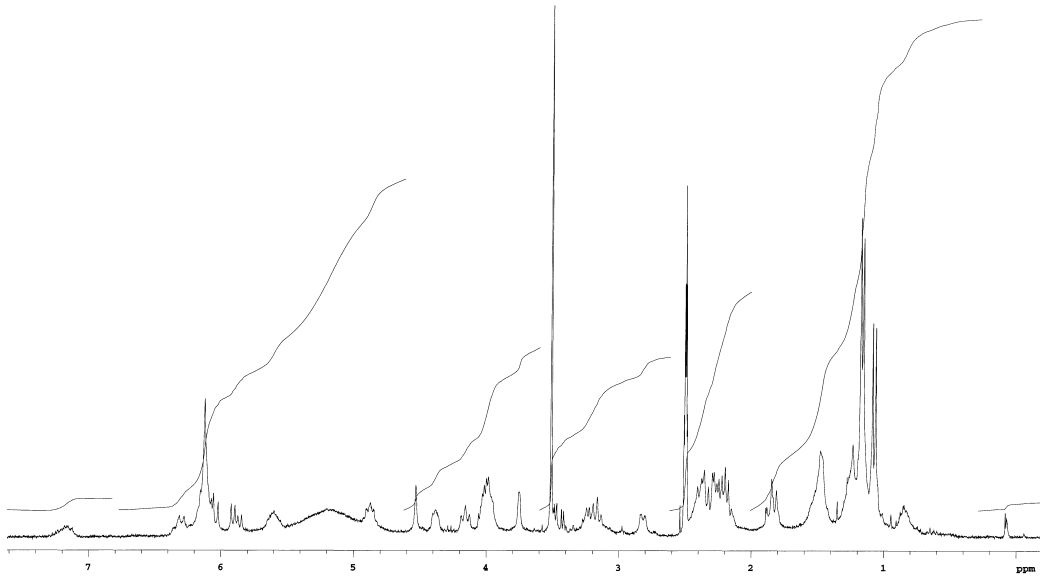


Figure 178. ^{1}H NMR spectrum (DMSO-d_6), 300 MHz] of CE-108D (**120d**).

In the ^{13}C NMR spectrum, 36 carbon signals were observed, which is in agreement with the HRESI mass spectrum. These carbon signals could be classified as three carbonyls: one ketone CO at δ 208.6, two CO signals corresponding to an acid, amid or ester at δ 173.0 and 176.4. Eight sp^2 carbon signals were in the range of δ 136.4-128.5, two anomeric carbons gave signals at δ 96.9 (C_q) and δ 95.9 (CH). Also oxygenated carbons were observed between δ 74.1 to 65.5, which were attributed to C-3, 9, 13, 15, 17, 27, 2', 4' and 5'. Finally three methyls were present at δ 20.2, 17.8, and 13.2, respectively.

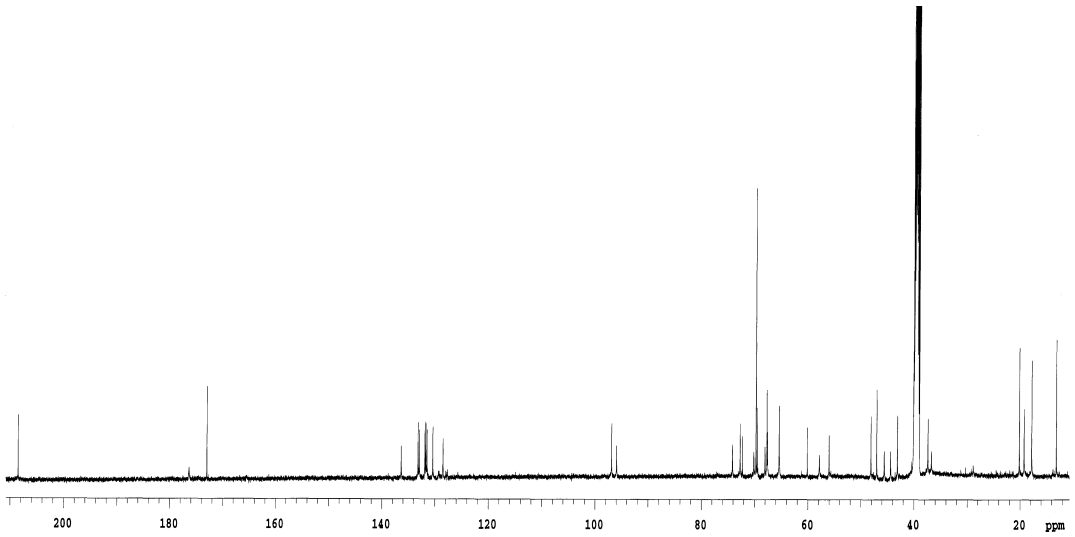


Figure 179. ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of CE-108D (**120d**).

To confirm the structure of CE-108D (**120d**), it was subjected to 2D NMR measurements. The H,H COSY spectrum experiment showed a correlation series beginning with the methine carbinol at δ 4.38, assigned to H-17, which was coupled with one of the methylene protons at δ 2.16 assigned to H-16 and with the sp^2 methine doublet of doublet at δ 5.89 (H-18). The latter proton correlated with H-19 at δ 6.06, which in turn coupled with another sp^2 methine proton H-20 at δ 6.32. The signal at δ 2.81 (H-3') was correlated to the proton at δ 3.75 (H-2') and also with the methine proton at δ 3.16 (H-4'), which correlated to another methine at δ 3.24 to construct a part of the sugar moiety. The methylene protons at δ 2.15 and 1.53 assigned to H-16 coupled with the carbinol methine at δ 4.16 (H-15). The H,H COSY and HMBC correlation confirmed the southern hemisphere and the structure of the amino sugar clearly. The anomeric proton H-1' (4.53) exhibited a ^3J correlation with C-17 (74.1), which confirmed the position of the sugar.

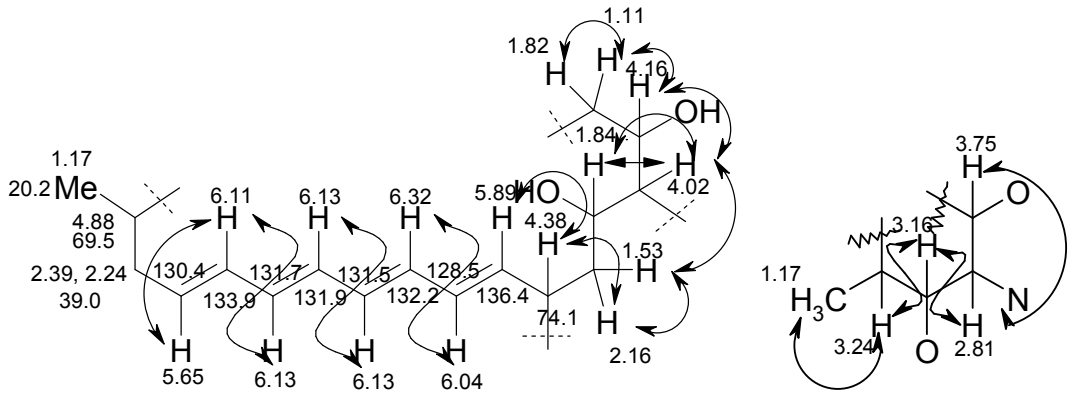


Figure 180. Selected correlations observed in the H,H COSY spectra of CE-108D (**120d**).

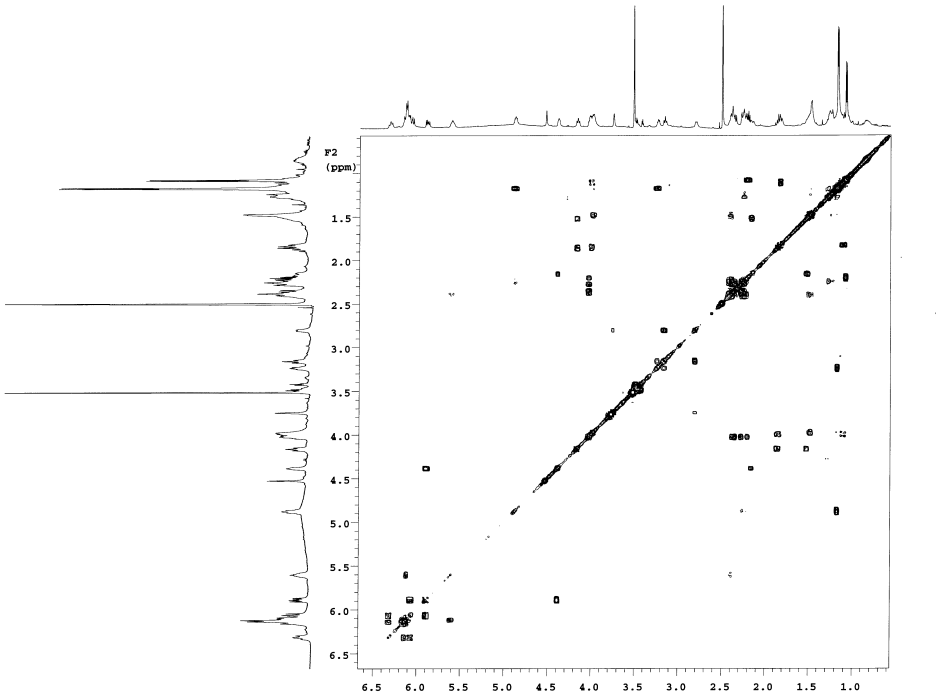


Figure 181. H,H COSY spectrum (DMSO-d_6, 600 MHz) of CE-108D (**120d**).

The interpretation of couplings in the northern part of the molecule was difficult, due to strong signal overlapping. The HMBC ^3J coupling of the proton at δ 4.88 (C-27) to the carbonyl at δ 173.0 (C-1) confirmed the lactone. The methyl protons of 2-Me displayed ^3J couplings with the lactone carbonyl, the carbinol carbon C-3 (67.7) and a ^2J coupling with C-2 (47.0). The methyl protons (CH_3-2) showed an additional COSY correlation with the methine at δ 2.20 (H-2), which in turn correlated to the carbinol methine at δ 4.03 (H-3). The HMBC correlations confirmed those observed in the COSY spectrum, which led to complete elucidation of the structure of CE-108D as **120d**. It should be mentioned, however, that there was no COSY or HMBC correlation between C-8 and C-9 or, respectively, their hydrogens in both **120d** and **120e**.

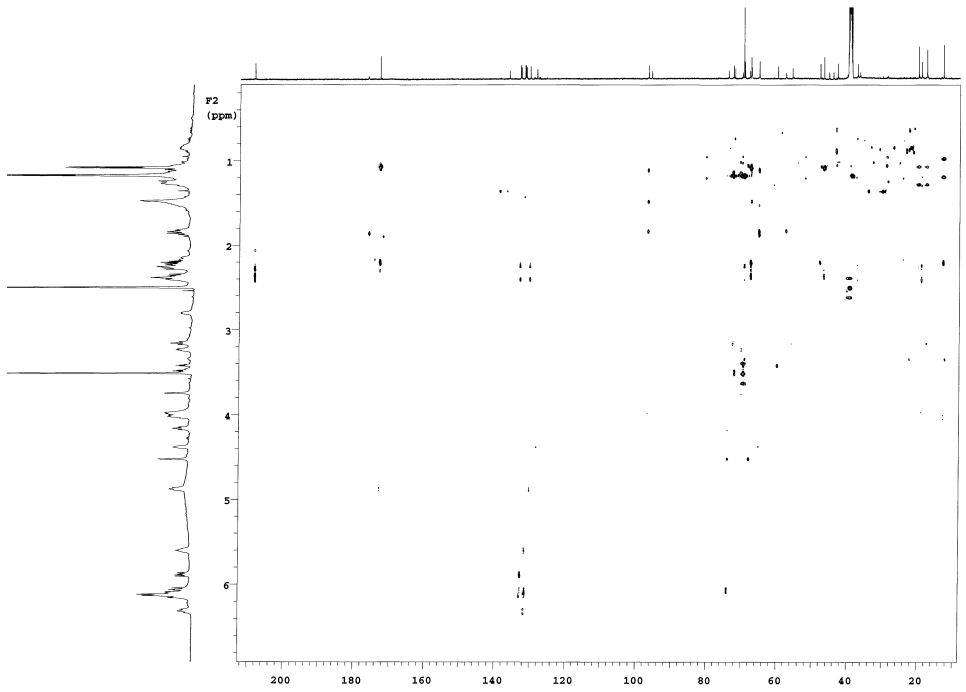


Figure 182. HMBC spectrum (DMSO-d_6, 600 MHz) of CE-108D (**120d**).

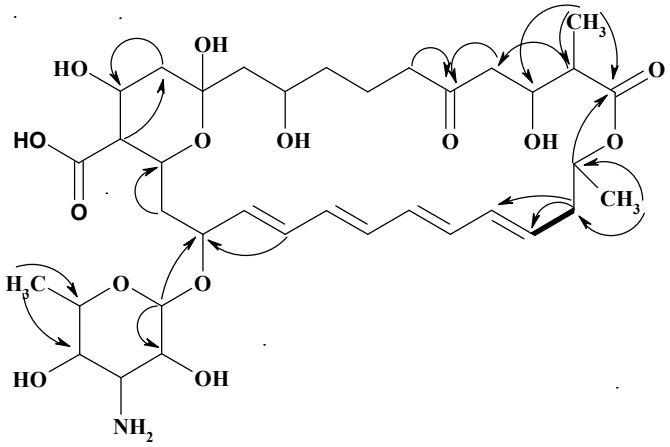


Figure 183. Selected HMBC correlations of CE-108D (**120d**).

6.8.2 CE-108E

Compound CE-108E (**120e**) was obtained as a pale yellow powder and showed a typical tetraene UV spectrum with λ_{max} at 317, 302 and 287 nm similar to that of **120d**.[228] The combined data of HRESIMS, ^{13}C NMR and ^{1}H NMR of CE-108E delivered the molecular formula $C_{36}H_{56}N_2O_{13}$. The ^{1}H NMR spectrum was very similar to that of CE-108D (**120d**). By comparing the molecular formula of CE-108E (**120e**) and CE-108D (**120d**), the former one must have an amide group ($CONH_2$) instead of the carboxylic group (COOH) in the latter one. The 2D NMR experiments showed the same correlations like CE-108D (**120d**), so that the structure **120e** is fully confirmed.

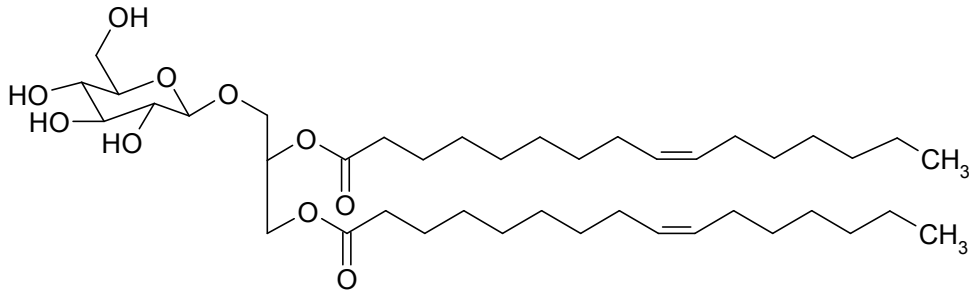
Table 16. ^{1}H NMR and ^{13}C NMR Data of CE-108D (**120d**) and CE-108E (**120e**) in DMSO-d_6.

	CE-108D (**120d**)		CE-108E (**120e**)	
Position	^{1}H (Int., mult, *J* [Hz])	^{13}C	^{1}H (Int., mult., *J* [Hz])	^{13}C
1	-	173.0	-	173.0
2	2.20 (1H, m)	47.0	2.20 (1H, m)	47.0
2-Me	1.08 (3H, d, 10.8)	13.2	1.08 (3H, d, 7.0)	13.1
3	4.03 (1H, m)	67.7	4.03 (1H, m)	67.8
4	2.36, 2.28 (2H, m)	48.1	2.36, 2.30 (2H, m)	48.1
5	-	208.5	-	208.6
6	2.39, 2.24 (2H, m)	43.1	2.43, 2.24 (2H, m)	43.1
7	1.49, 1.26 (2H, m)	19.3	1.53, 1.28 (2H, m)	19.3
8	1.28, 1.20 (2H, m)	37.4	1.28, 1.20 (2H, m)	37.4
9	3.98 (1H, m)	67.6	3.98 (1H, m)	67.6
10	1.48 (2H, m)	45.6	1.45 (2H, m)	45.6
11	-	96.9	-	96.9
12	1.82, 1.11 (2H, m)	44.4	1.89, 1.12 (2H, m)	44.7
13	4.00 (1H, m)	65.4	4.02 (1H, m)	64.7
14	1.84 (1H, m)	57.0	1.92 (1H, t, 10.3)	56.6
CONH	-	176.4	-	174.2
15	4.16 (1H, t, 8.4)	65.4	4.17 (1H, t, 9.6)	65.2
16	2.16, 1.53 (2H, m)	36.7	2.06, 1.51 (2H, m)	36.6
17	4.38 (1H, m)	74.1	4.37 (1H, m)	74.4
18	5.89 (1H, dd, 15.2, 8.2)	136.4	5.87 (1H, dd, 15.3, 8.4)	136.3
19	6.06 (1H, dd, 15.2, 10.7)	128.5	6.06 (1H, m)	128.5
20	6.32 (1H, m)	132.2	6.31(1H,dd, 13.9, 10.7)	132.9
21	6.13 (1H, m)	131.5	6.13 (1H, m)	131.2
22	6.13 (1H, m)	131.9	6.13 (1H, m)	131.9
23	6.13 (1H, m)	131.7	6.13 (1H, m)	131.8
24	6.11 (1H, m)	133.9	6.13 (1H, m)	133.2
25	5.60 (1H, m)	130.4	5.61 (1H, m)	130.4
26	2.39, 2.24 (2H, m)	39.0	2.41, 2.29 (2H, m)	39.0
27	4.88 (1H, m)	69.5	4.88 (1H, m)	69.5
28	1.17 (3H, d, 6.1)	20.2	1.16 (3H, d, 6.7)	20.2
Sugar				
1'	4.53 (1H, s)	95.9	4.39 (1H, s)	96.4
2'	3.75 (1H, d, 1.7)	68.0	3.69 (1H, d, 1.6)	68.5
3'	2.81 (1H, d, 4.7)	56.0	2.62 (1H, m)	56.0
4'	3.16 (1H, dd, 9.6, 8.9)	70.1	3.06 (1H, m)	70.9
5'	3.24 (1H, m)	72.7	3.12 (1H, m)	72.9
6'	1.17 (3H, d, 6.1)	17.8	1.16 (3H, d, 6.7)	17.8
OH/N	7.18, 5.20 (brs)	-	7.32, 6.83 (brs)	-

7 Summary

Natural products have provided the major sources of chemical diversity for pharmaceutical discoveries for many decades. For clinical application to treat human diseases in almost all therapeutic areas, many natural products and synthetically modified natural products have been successfully used. In the search for novel and bioactive molecules for discovery of new drugs, marine-derived natural resources are becoming an important research area.

The investigation of a Jordanian *Scytonema* strain resulted in 3-O-β-D-galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)glycerol (**33**), diglycolipid 16 (**35**) and oleic acid (**36**). In addition, the MALDI-TOFMS analysis showed the presence of pheophytin a (**31**) and pheophobide a (**32**). Diglycolipid 16 (**35**) was found to inhibit HIV-1-RT enzymatic activity.[37] Oleic acid is the main monounsaturated fatty acid of olive oil and showed anticancer activity against breast cancer cells.[38] Furthermore, it was reported that oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells.[39]



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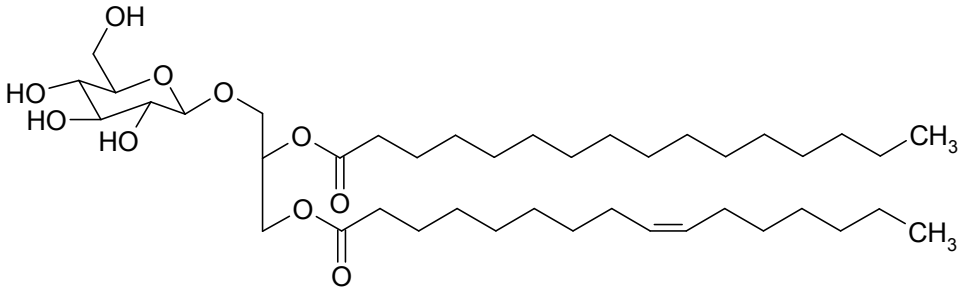
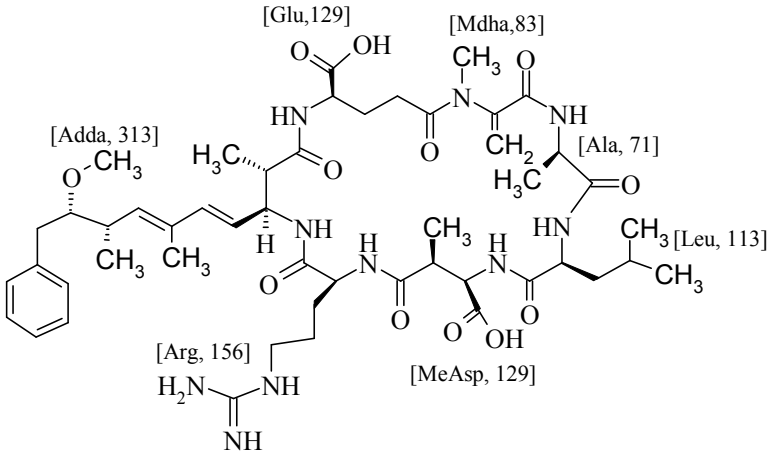


35

Microcystis aeruginosa is another strain of cyanobacteria which was collected from King Talal Dam in Jordan. Two hepatotoxic cyclic heptapeptide microcystin LR (**37**) and microcystin YR (**39**) were isolated from *Microcystis aeruginosa* and identified through HPLC-ESI-MS/MS from this strain. Also, it was reported that the amino acids Adda plays a major role for the bioactivity of these microcystins.[41,42]



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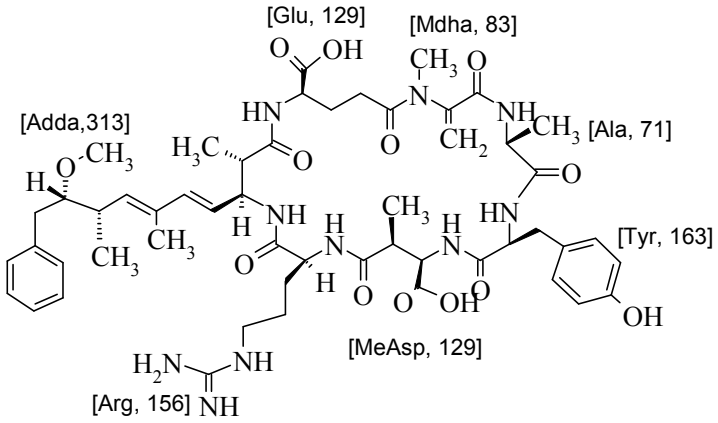
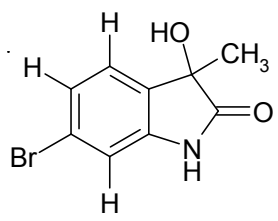


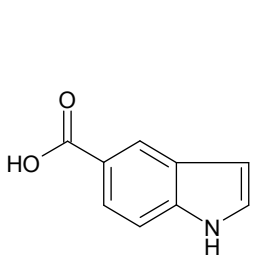
39

Bromoanaindolone (**42**) is a new racemic secondary metabolite isolated from the cyanobacterium *Anabaena constricta*; it showed antibacterial activity against the Gram-positive bacterium *Bacillus cereus* at MIC 128 µg ml^{-1}.



42

The marine-derived Streptomyces sp. B7801 showed highly antibacterial activity against *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57) as well as antifungal activity against *Mucor miehei* (Tü 284). Purification of the crude extract obtained from a 25 L shaker culture led to five new compounds, namely indole-5-carboxylic acid (**43**), ramthacin A (**44**), B (**47**), C (**48**) and 4-(5-hydroxy-3,4-dimethy-l-pent-3-enyl)-5*H*-furan-2-on (**49**).



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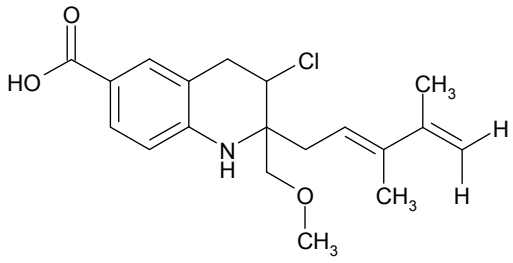

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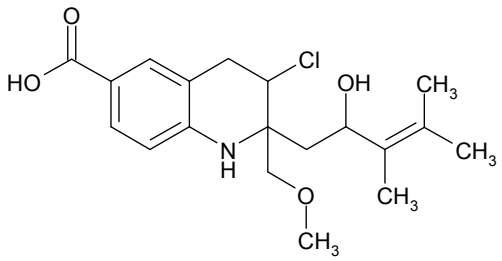

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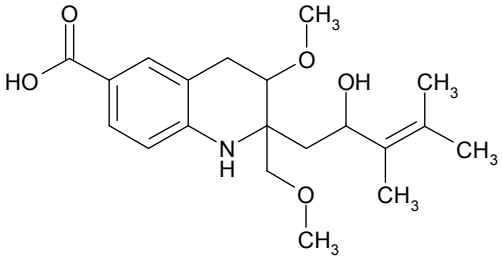
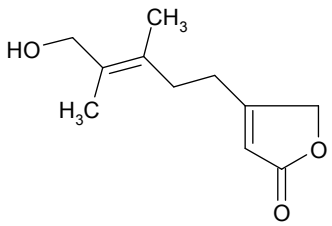
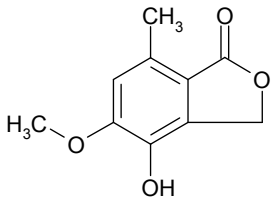




48 **49**

The crude extract of the marine *Streptomyces* sp. B8041 strain showed only weak activity against *Staphylococcus aureus*. In the chemical screening of the crude extract, a moderately polar zone, which turned green with anisaldehyde/sulphuric acid on TLC, delivered two new compounds, namely MR2621C (**61**) and 4-hydroxy-5-methoxy-7-methy-3*H*-isobenzofuran-1-one (**56**) along with four known compounds, saptomycin A (**58**), *β*-indomycinone (**57**), and a mixture of two diketopiperazines. The new compounds were antibiotically inactive and showed also no cytotoxicity.



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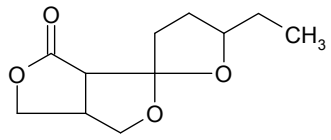

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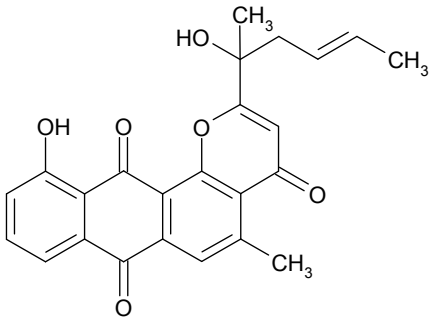

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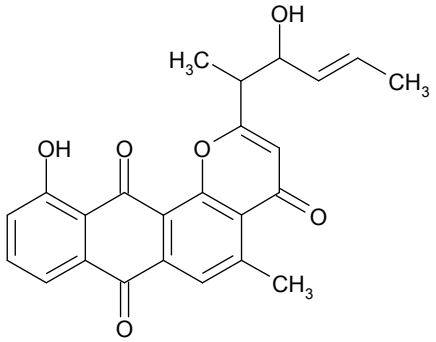
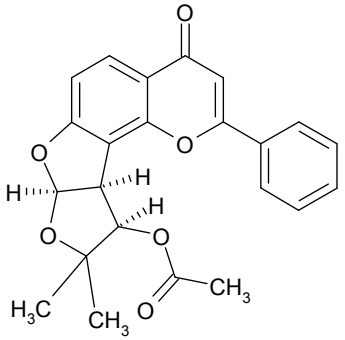


58

In the agar diffusion test, the marine-derived *Streptomyces* sp. B8013 was found to inhibit the growth of *Mucor miehei* (Tü284), *Candida albicans* and *Staphylococcus aureus*. From a 25 L shaker culture, a total of 19 compounds were isolated. Among them, two compounds were firstly isolated from marine source: pseudosemiglabrin (**72**) and semiglabrin (**73**) and one new butenolide, 4,9,10-trihydroxy-10-methyldodec-2-en-1,4-olide (**79**).



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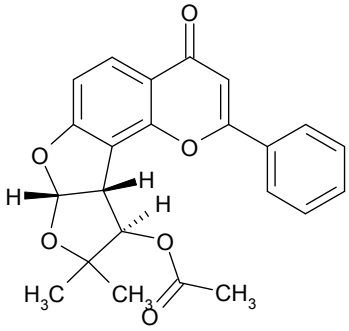

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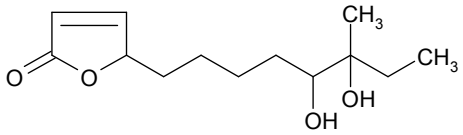
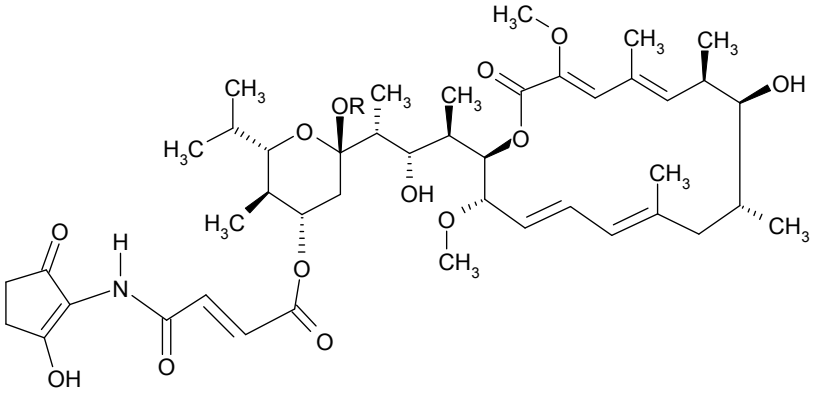


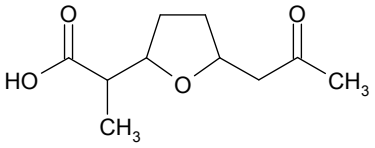
79

The terrestrial streptomycete isolate GT 2005/014 exhibited moderate activity against *Streptomyces viridochromogenes* (Tü 57) and *Mucor miehei* (Tü 284) but no activity against other bacteria tested and 92% activity in the brine shrimp test. HPLC

MS of the crude extract indicated the presence of bafilomycin B_1 (**85a**) and B_2 (**85b**) as well as one new compound named attiamycin B (**90**), which has moderate antibacterial activity against *Staphylococcus aureus*.

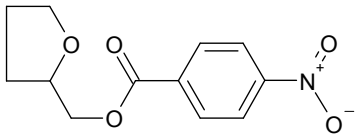


85a: R = H Bafilomycin B_1 **85b:** R = Me Bafilomycin B_2



90

From the terrestrial *Streptomyces* Wo 990 strains, two new compounds were isolated namely, 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**) and 4-hydroxy-5-methylfuran-3-one (**98**). The former one showed activity in the agar diffusion test against the micro algae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus* as well as 54 % cytotoxicity against brine shrimps.



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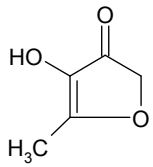
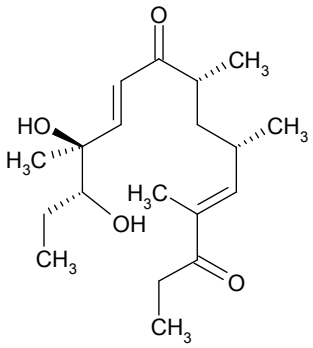


98

The terrestrial *Streptomyces* sp. Ank22 produced a variety of kromycin derivatives, namely *seco*-decarboxy-kromycin (**105**), 10,11-dihydro-kromycin (**106**), kromycin (**107**), a mixture of neomethymycin (**108**) and methymycin (**109**) and picromycin (**110**). Among them, **105** and **106** were new.



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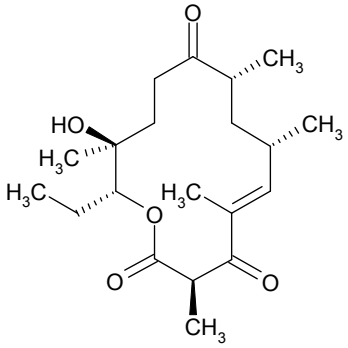
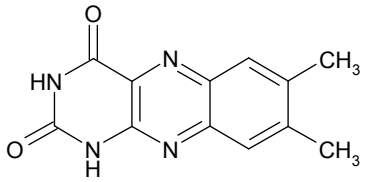


106

The terresterial *Streptomyces* sp. Ank123 was selected due to its biological activity against *Streptomyces viridochromogenes* (Tü57) and weak activity against *Escherichia coli* and *Bacillus subtilis*. From this strain, lumichrome (**111**), indole-3-carbonyl-L-rhamnopyranoside (**112**), 3-butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**) and monensin B (**119**) were isolated; 6-methoxy-6-propyl-tetrahydro-furo[3,4,-c]furan-1-one (**117**) is a new natural product.



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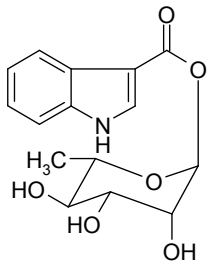

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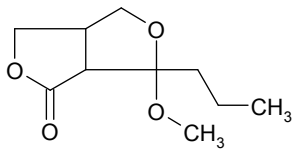

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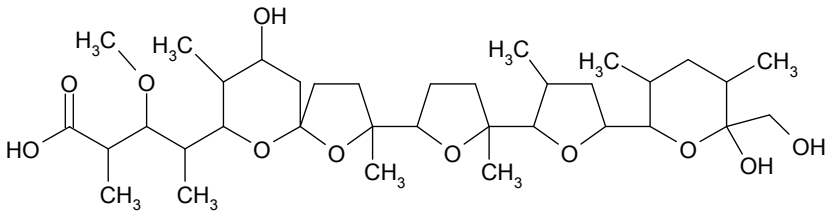

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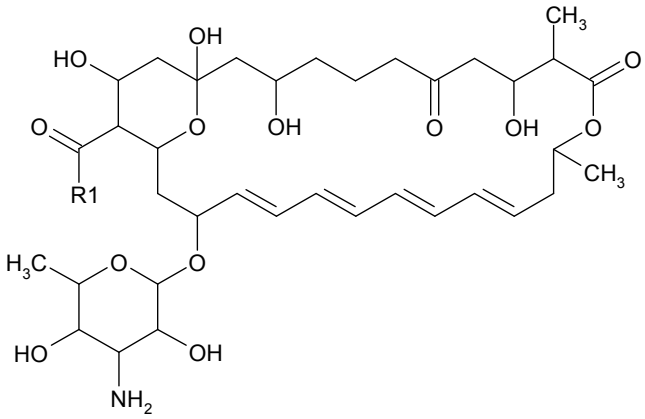
Most of the polyene macrolides have been reported to have selective antifungal activity and some of them were modified by attaching the sugar moiety to increase the poor water solubility. Both the carboxy group on the side chain and amino group on sugar moiety were epected to have the influence on the biological properties of

these compounds. The two new polyene macrolides CE-108D and CE-108E were produced from genetically modified bacteria *Streptomyces diastaticus* var. 108.



120d CE-108D R = OH **120e** CE-108E R= NH_2

In this work three cyanobacteria, six marine-derived bacteria and eight terrestrial *Streptomycetes* strains were studied. From the cyanobacteria seven compounds were identified, from which one is a new secondary metabolites. The six marine strains were found to produce twenty-eight compounds, out of which eleven are reported here for the first time. From the terrestrial strains twenty-seven secondary metabolites were isolated and characterised including nine new compounds. In total, 62 compounds were isolated consisting of different classes of compounds such as peptides, quinones, and butenolides.

Table 17. Total number of isolated compounds from bacteria in this thesis.

Strains	No of strains	No of compounds	No of new compounds
Cyanobacteria	3	7	1
Marine *Streptomycetes*	6	28	11
Terrestrial *Streptomycetes*	8	27	9

8 Experimental Part

8.1 General

IR spectra: Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 Infrared Spectrophotometer; Beckman DU-640; Shimadzu FT-IR; (KBr tablet and film). – UV/VIS spectra: Perkin-Elmer Lambda 15 UV/VIS spectrometer. – Optical rotations: Polarimeter (Perkin-Elmer, model 241). – ^{1}H NMR spectra: Varian Unity 300 (300.542 MHz), Bruker AMX 300 (300.542 MHz), Varian Inova 500 (499.8 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. – ^{13}C NMR spectra: Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethylsilane as internal standard. Abbreviations: APT (Attached Proton Test): CH/CH_3 up and C_q/CH_2 down. - 2D NMR spectra: H,H COSY spectra (^{1}H,^{1}H-Correlated Spectroscopy), HMBC spectra (Heteronuclear Multiple Bond Connectivity), HMQC spectra (Heteronuclear Multiple Quantum Coherence) and NOESY spectra (Nuclear Overhauser Effect Spectroscopy). - Mass spectra: EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosene as standard. ESIMS with Quattro Triple Quadruple mass spectrometer Finigan MAT-Incos 50, ESIMS LCQ (Finnigan).

8.2 Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). – Glass plates for chemical screening: Merck silica gel 60 F254, (10 × 20 cm). - Preparative thin layer chromatography (PTLC): 55 g silica gel P/UV_{254} (Macherey-Nagel & Co.) is added to 120 ml of demineralised water with continuous stirring for 15 minutes. 60 ml of the homogenous suspension is poured on a horizontal held (20 × 20 cm) glass plate and the unfilled spaces are covered by distributing the suspension. The plates are air dried for 24 hours and activated by heating for 3 hours at 130 °C. - Column chromatography (CC): MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co). Sephadex LH-20 (Pharmacia) was used for size exclusion chromatography.

8.3 Spray reagents

Anisaldehyde/sulphuric acid: 1 ml anisaldehyde was added to 100 ml of a stock solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid. – Ehrlich's reagent: 1 g 4-dimethylaminobenzaldehyde was dissolved in a mixture of 25 ml hydrochloric acid (37%) and 75 ml methanol; it gives a red to violet colouration with

indoles, a yellow colour with anthranilic acid derivatives and a grey colour reaction with some N-heterocycles. – Ninhydrin: 0.3 g ninhydrin (2,2-dihydroxyindan-1,3-dione) was dissolved in 95 ml *iso*-propanol. The mixture was added to 5 ml collidin (2,4,6-trimethylpyridin) and 5 ml acetic acid (96%). This reagent gives a blue to a violet colouration with amino acids, peptides and polypeptides with free amino groups.

Chlorine/*o*-dianisidin reaction: The reagent was prepared from 100 ml *o*-dianisidin (0.032%) in 1 N acetic acid, 1.5 g $Na_2WO_4 \cdot 2\ H_2O$ in 10 ml water, 115 ml acetone and 450 mg KI. The moistened TLC plate was kept ca. 30 min in a chlorine atmosphere (from 0.5 g $KClO_3$ + 2 ml conc. HCl) and then dried for ca. 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent.

NaOH or KOH: 2 N NaOH or KOH solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from orange to violet or blue.

8.4 Microbiological materials

Storage of strains: Deep-freeze storage in a Dewar vessel, 1'Air liquid type BT 37 A. - Capillaries for deep-freeze storage: diameter 1.75 mm, length 80 mm, Hirschmann Laborgeräte Eberstadt. – Soil for soil culture: Luvos Heilerde LU-VOS JUST GmbH & Co. Friedrichshof (from the health shop). - Ultraturrax: Janke & Munkel KG. – Shaker: Infors AG (CH 4103 Einbach) type ITE. - Laboratory shaker: IKA-shaker type S50 (max. 6000 Upm). - Autoclave: Albert Dargatz Autoclave volume 119 l, working temperature 121 °C, working pressure 1.2 kg/cm^2. - Antibiotic assay discs: 9 mm diameter, Schleicher & Schüll No. 321 261. - Culture media: glucose, bacto peptone, bacto agar, dextrose, soybean, mannitol, yeast extract and malt extract were purchased from Merck, Darmstadt. - Petri-dishes: 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – Celite: Celite France S. A., Rueil-Malmaison Cedex. - Sterile filters: Midisart 2000, 0.2 µm, PTFE-Filter, Sartorius, Göttingen. - Laminar-Flow-Box: Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. - Brine shrimp eggs (*Artemia salina*): SERA Artemia Salinenkrebseier, SERA Heinsberg. - Salinenkrebsfutter: micro cell DOHSE Aquaristik KG Bonn (brine shrimp eggs and food can be obtained from aquaristic shops).

8.5 Recipes

All cultures media were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1 L shaker culture: 33 min.

Artificial sea water

Iron citrate	2 g (powder)
NaCl	389 g
$MgCl_2 \cdot 6\ H_2O$	176 g
Na_2SO_4	68.8 g
$CaCl_2$	36.0 g
Na_2HPO_4	0.16 g
SiO_2	0.30 g
Trace element stock soln.	20 mL
Stock soln.	200 mL
tap water	add 20 L

Trace element stock solution

H_3BO_3	0.611 g
$MnCl_2$	0.389 g
$CuSO_4$	0.056 g
$ZnSO_4 \cdot 7\ H_2O$	0.056 g
$Al_2(SO_4)_3 \cdot 18\ H_2O$	0.056 g
$NiSO_4 \cdot 6\ H_2O$	0.056 g
$CO\ (NO_3)_3 \cdot 6\ H_2O$	0.056 g
TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	0.056 g
LiCl	0.028 g
$SnCl_2$	0.028 g
KI	0.028 g
tap water	ad 1 L

Stock solution

KCl	110 g
$NaHCO_3$	32 g
KBr	16 g
$SrCl_2 \cdot 6H_2O$	6.8 g (dissolved separately)
H_3BO_3	4.4 g
NaF	0.48 g
NH_4NO_3	0.32 g
tap water	ad 2 L

8.5.1 Nutrients

M_2 medium (without sea water)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Tap water	ad 1 L

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar

M_2^+ medium (M_2 medium with sea water)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Artificial seawater	500 mL
Tap water	500 mL

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

Luria-Bertani-Medium (LB)

Trypton	10 g
Yeast extract	5 g
NaCl	10 g
Tap water	1000 mL

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

Soybean-Mannitol Medium

Soybean meal (defatted)	20 g
D (-)-Mannitol	20 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

M Test Agar (for test organisms Escherichia coli, Bacillus subtilis (ATCC 6051), Staphylococcus aureus, Mucor miehei (Tü 284):

Malt extract	10 g
Yeast extract	4 g
Glucose	4 g
Bacto agar	20 g
Demineralised water	1000 mL

The pH was adjusted to 7.8 using 2N NaOH.

Sabouraud-Agar

(for test organism *Candida albicans*)

Glucose	40 g
Bacto peptone	10 g
Bacto agar	20 g
Demineralised water	1000 mL

The pH was adjusted to 7.8 using 2N NaOH.

Nutritional solution A

Soybean meal (defatted)	30 g
Glycerol	30 g
$CaCO_3$	2 g
Artificial sea water	750 ml
Demineralised water	250 ml

Nutritional solution B

Starch	10 g
NZ-Amine	5 g
Soybean meal	2g
Yeast extract	5 g
KNO_3	3 g
Algal extract	2.5 ml
Artificial sea water	750 ml
Demineralised water	250 ml

Stock solutions and media for cultivation of algae

Fe-EDTA

0.7 g of $FeSO_4 \cdot 7\ H_2O$ and 0.93 g EDTA (Titriplex III) are dissolved in 80 ml of demineralised water at 60 °C and then diluted to 100 ml.

Trace element Solution II:

Solution A:

$MnSO_4 \cdot H_2O$	16.9 mg
$Na_2MoO_4 \cdot 2H_2O$	13.0 mg
$Co\ (NO_3)_2 \cdot 6H_2O$	10.0 mg

Salts are dissolved in 10 ml of demineralised water.

Solution B:

$CuSO_4 \cdot 5H_2O$	5.0 mg
H_3BO_3	10.0 mg
$ZnSO_4 \cdot 7H_2O$	10.0 mg

Salts are dissolved each in 10 ml of demineralised water. Solutions A is added to B and diluted to 100 ml with demineralised water.

Bold's Basal medium (BBM): (for algae Chlorella vulgaris, Chlorella sorokiniana and Scenedesmus subspicatus.

$NaNO_3$	0.250 g
KH_2PO_4	0.175 g
K_2HPO_4	0.075 g
$MgSO_4 \cdot 7\ H_2O$	0.075 g
NaCl	0.025 g
$CaCl_2 \cdot 2\ H_2O$	0.025 g
Fe-EDTA	1.0 ml
Trace element solution II	0.1 ml

Salts are dissolved in 10 ml of demineralised water and added to Fe-EDTA and trace element solution II. The mixture made to one litre with demineralised water. Solid medium was prepared by adding 18 g of agar. Bitte Zeilenabstand kontrollieren!

Chu's 10 Medium Modified

$Ca(NO_3)_2 \cdot 4H_2O$	0.232 g
K_2HPO_4	0.01 g
$MgSO_4 \cdot 7H_2O$	0.025 g
Na_2CO_3	0.02 g
$Na_2SiO_3 \cdot 5H_2O$	0.044 g
Ferric citrate	3.5 mg
Citric acid	3.5 mg
Agar	15.0 g
Metal Solution	1.0 ml
Distilled water	1.0 L

Metal Solution:

H_3BO_3	2.4 g
$MnCl_2 \cdot 4H_2O$	1.4 g
$ZnCl_2$	0.4 g
$CoCl_2 \cdot 6H_2O$	0.02 g
$CuCl_2 \cdot 2H_2O$	0.1 mg
Distilled water to	1.0 L

8.6 Microbiological and analytical methods

8.6.1 Storage of Strains

All bacteria strains were stored in liquid nitrogen for long time. The strains were used to inoculate agar plates with the suitable media at room temperature.

8.6.2 Pre-Screening

The microbial isolates (obtained from culture collections) were cultured in a 1 L scale in 1 L-Erlenmeyer flasks each containing 200~250 ml of M_2 or (for marine strains) M_2^+ medium. The flasks were shaken for 3-5 days at 28 °C, after which the entire fermentation broth was freeze-dried and the residue extracted with ethyl acetate. The extracts were evaporated to dryness and used for the antimicrobial tests in a concentration of 50 µg/ml.

8.6.3 Biological screening

The crude extract was dissolved in $CHCl_3$/10% MeOH (concentration 50 µg/mL), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on an agar plates inoculated with *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Candida albicans* and *Mucor miehei* (Tü 284).

The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungi (24 hours), and 24-26 °C under day-light for micro-algae (96 hours). The diameter of the inhibition zones was measured by ruler.

8.6.4 Chemical and pharmacological screening

Samples of the extracts were separated on silica gel glass plates (10 × 20 cm) with two solvent systems $CHCl_3$/5% MeOH and $CHCl_3$/10% MeOH. After drying, the plates were photographed under UV light at 254 nm and marked at 366 nm, and sub-

sequently stained by anisaldehyde and Ehrlich's reagent. Finally, the plates were scanned for documentation. For the pharmacological investigations, approximately 25 mg of the crude extract was sent to industrial partners.

8.6.5 Brine shrimp microwell cytotoxicity assay

To a 500 ml separating funnel, filled with 400 ml of artificial seawater, 1 g of dried eggs of *Artemia salina* and 1 g food were added. The suspension was aerated by bubbling air into the funnel and kept for 24 to 48 hours at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. 30 to 40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 ml of salt water and the dead larvae counted (number N). A solution of 20 µg of the crude extract in 5 to 10 µl DMSO was added and the plate kept at r.t. in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of ca. 0.5 ml methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in %. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 µg/test actinomycin D. The mortality rate M was calculated using the following formula:

$$M = \left[\frac{(A - B - N)}{(G - N)} \right] \cdot 100$$

with

M = percent of the dead larvae after 24 h.
A = number of the dead larvae after 24 h.
B = average number of the dead larvae in the blind samples after 24 h
N = number of the dead larvae before starting of the test.
G = total number of brine shrimps

8.6.6 Primary screening

Antibiotic screening (disk diffusion test): The test is performed using paper discs with a diameter of 8 mm under standardized conditions. If the inhibition zone is ranging from 11 to 20 mm, the compound is considered to be weakly active (+), from 21 to 30 mm designated as active (++) and over 30 mm is highly active (+++). - Chemi-

cal screening: evaluation of the separated bands by the number, intensity and colour reactions with different staining reagents on TLC. - Toxicity test: By counting survivors after 24 hrs, the mortality of the extracts was calculated (see above). The extracts, fractions or isolated compounds were considered inactive when the mortality rate was lower than 10% (-), from 10 to 59% as weakly active (+), from 60 to 95% as active (++) and over 95% as strongly active (+++).

9 Metabolites from selected strains

9.1 Scytonema

9.1.1 General Isolation Procedure.

The *Scytonema* strain was collected by Prof. Alani, isolated by Prof. Al-Mahasnih from Al al-Bayt University, and identified by AnagnosTec GMbH, Luckenwalde, Germany. Freeze-dried bacterial cells were first extracted with 1:1 CH_2Cl_2/MeOH (3 x 0.5 L) and then with MeOH (3 x 0.5 L). The filtered extracts were combined and concentrated under reduced pressure. The crude extract was chromatographed over silica gel column, eluted from the column with solvents of increasing polarity: CH_2Cl_2/MeOH, 100:0, 99:1, 98:2, 95:5, 9:1, 8:2, 7:3, 1:1, 0:100. Guided by TLC, fractions were combined to afford three main fractions. Fraction I was loaded on a silica gel column eluted with cyclohexane/ethyl acetate to obtain oleic acid (**36**, 10.5 mg). Purification of fraction II using silica gel eluted with CH_2Cl_2/5% MeOH followed by Sephadex LH-20 eluted with methanol resulted in a mixture (11.2 mg) of 3-O-β-D-galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)–glycerol (**33**) and diglycolipid 16 (**35**).

3-O-β-D-Galactopyranosyl-1,2-di-O-(9Z-hexadecenoyl)glycerol (33) / Diglycolipid 16 (35): Weak UV absorbing (254 nm), colourless oil, black colouration with anisaldehyd reagent. – **^{1}H NMR** (300 MHz, CD_3OD) δ 5.35 (t, ^3J = 12.0, 2H, H-9', 10'), 5.30 (m, 1H, H-2), 4.47 (dd, 1H, ^2J = 3.2, ^3J = 12.0 Hz, H-1a), 4.21 (dd, 1H, ^2J = 6.4, ^3J = 12.0 Hz, H-1b), 3.98 (dd, 1H, ^2J =5.4, ^3J = 11.2 Hz, H-3a), 3.73 (dd, 1H, ^2J = 6.4, ^3J =11.2 Hz, H-3b), 4.22 (d, 1H, ^3J = 7.5 Hz, H-1'''), 3.73 (m, 1H, H-2'''), 3.52 (dd, 1H, ^3J = 9.2, ^2J = 3.27 Hz, H-3'''), 3.82 (d, ^3J = 3.2 Hz, 1H, H-4'''), 4.20 (m, 1H, H-5'''), 3.75 (m, 1H, H-6a'''), 3.48(m,1H, H-6b'''), 2.33 (td, ^3J = 6.8, ^2J = 3.4 Hz, 4H, H-2', 2''), 2.00 (m, 4H, H-8'', 10''), 1.60 (m, 4H, H-3', 3'') 1.40-1.22 (40H, m, H-4'', H-14'', H-4'-H-7', H-11'- H-14'), 0.88 (t, 6H, ^3J = 7.0 Hz, H-15', H-15''). – **(+)-ESIMS** *m/z* 1479 ([2 M1+ Na]$^+$, 45), 751 ([M_1+ Na]+, 100), 1475.4 ([2 M_2+ Na]$^+$, 35), 749.6 ([M_2+ Na]$^+$, 100), 1477.4 ([M_1+ M_2 + Na], 60). – **(+)-ESIHRMS** *m/z* 744.56215 (calcd $C_{41}H_{74}O_{10}NH_4$, 744.56254).

9.2 Microcystis aeruginosa

9.2.1 Isolation and Purification.

The *Microcystis aeruginosa* strain investigated here was isolated and identified by Prof. S. Al-Jasabi and Prof. A. Khalil from Yarmouk university. The strain was collected from king Talal dam by Dr. M. Halawa Pheladelphia university, Jordan. The air dried powdered cell material of *Microcystis aeruginosa* (50.0 g) was chopped and macerated (1.5 L × 3) for 2-day periods with dichloromethane/50% methanol at room temp. The solvent was evaporated under reduced pressure, to produce 50.0 g of crude extract. The crude extract was subjected to silica gel flash chromatography with cyclohexane, and the polarity of the solvent was gradually increased by addition of EtOAc to afford four main fractions. Fraction II was chromatographed over a silica gel column eluted with CH_2Cl_2/5% MeOH to provide palmitic acid and glycerol tricaprylate. In the same way fraction IV was subjected to Sephadex LH-20 column eluted with methanol to give a mixture of microcystin LR (**37**) and microcystin YR (**39**).

Microcystin LR (**37**) **:** UV absorbing, for the amino sequence see Table 1. **– (+)-ESIMS** *m/z* 995 $[M + H]^+$

Microcystin YR (**39**)**:** UV absorbing, for the amino sequence see Table 2. **– (+)-ESIMS** *m/z* 1045 $[M + H]^+$.

9.3 Cyanobacterium Anabaena constricta

The anticyanobacterial activity was determined in a special matrix assay described in detail earlier.[229] The assay is characterised by an overlay technique, namely by the application of a test compound on a porous matrix (silica gel) in well-defined quantities and of the subsequent coating of this matrix by a concentrated suspension of the living cyanobacterial test organism. This enables the detection of minimum toxic quantities of test compounds.

The antibacterial activity was determined according to the broth microdilution method. The assays were performed as serial dilution tests, and were carried out in plates as described previously.[230]

Bromoanaindolone (42): White solid; CD (MeOH): λ_{ext} ($[\theta]^{25}{}_{\%}$): 214 (+100), 238 (-78), 268 (+16); IR (KBr) ν_{max}: 3500-2800, 1734, 1612, 1062, 812 cm^{-1}. – ^1H NMR (300 MHz, DMSO-d_6). **– ^{13}C NMR** (75 MHz, DMSO-d_6), **– H,H COSY**, **HSQC** and **HMBC** data see **Table 3**. – EIMS (70 eV) *m/z* 241/243 ($[M]^+$ for ^{79}Br and ^{81}Br iso-

tope, 61/56), 226/228 ($[M-CH_3]^+$, 25/22), 213/215 ($[M-CO]^+$, 70/68), 198/200 ($[M-CONH]^+$, 100/94). – **(+)-HRESIMS** *m/z* 263.96303 $[M + Na]^+$), (calcd for $C_9H_8BrNO_2$, 263.96304).

9.4 Marine Derived *Streptomyces* sp. B7801

The strain *Streptomyces* sp. B7801 was isolated and identified by E. Helmke from the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. The strains formed a white mycelium after incubation on M_2^+ agar medium for 6 days at 28 °C.

9.4.1 Pre-Screening

The biological screening of the crude extract showed activity against *Staphylococcus aureus, Mucor miehei* and *Streptomyces viridochromogenes* (Tü57). The TLC showed UV absorbing bands, which gave green, and orange colour after spraying with anisaldehyde/sulphuric acid.

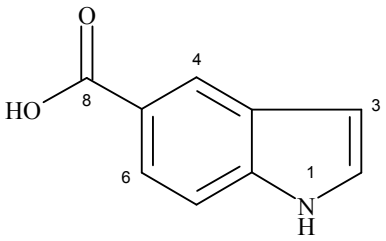
Table 18. Biological activity of the crude extract of marine *Streptomyces* sp. B7801.

Tested Microorganisms	Inhibition zone ∅ [mm]
Staphylococcus aureus	15
Streptomyces viridochromogenes (Tü57)	11
Mucor miehei (Tü 284)	18

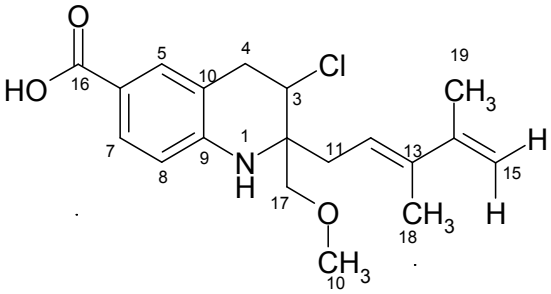
9.4.2 Fermentation, Extraction and Isolation:

The marine *Streptomyces* sp. isolate B7801 was pre-cultivated on M_2^+ agar plates (with 50% sea water) at 28 °C for 7 days. Pieces of a well grown agar subculture of the marine isolate B7801 were used for the inoculation of a 25 l shaker culture on M_2^+ medium. After 7 days of cultivation at 28 °C, a brown-yellow culture broth was obtained and filtered over Celite with the aid of the filter press. The filtrate was extracted using Amberlite XAD-16, then washed with methanol, while the mycelium was extracted with ethyl acetate followed by acetone. The biomass was extracted three times with ethyl acetate followed by acetone. Thin layer chromatography of the crude extracts from the water phase and the biomass showed identity, so they were combined. The crude extract (10.2 g), obtained after usual work-up from the 25 L fermentation, was separated over silica gel with a CH_2Cl_2/MeOH gradient to afford five fractions. Indole-5-carboxylic acid (**43**,3.9 mg) was obtained from fraction II by applying to Sephadex LH-20 (MeOH). Sephadex LH-20 of fraction III gave three sub-fractions. The sub-fractions IIIa and IIIc were subjected to silica gel column followed

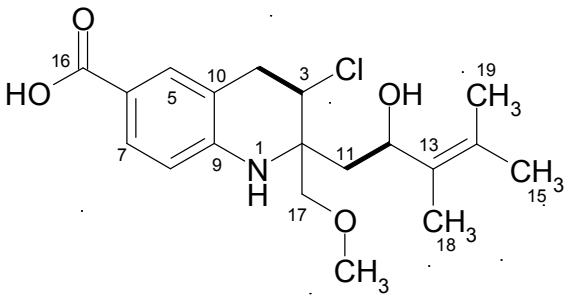
by PTLC to afforded ramthacin A (**44**, 1.1 mg), B (**47**, 1.3 mg) and C (**48**, 2.4 mg). Purification of sub-fraction IIIb yielded 4-(5-hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-on (**49**). Further fractionation and purification of fractions IV and V afforded the known compounds, tyrosol and xanthine (3,4,5,9-tetrahydro-purine-2,6-dione) (**52**, 1.1 mg) (Figure 23).



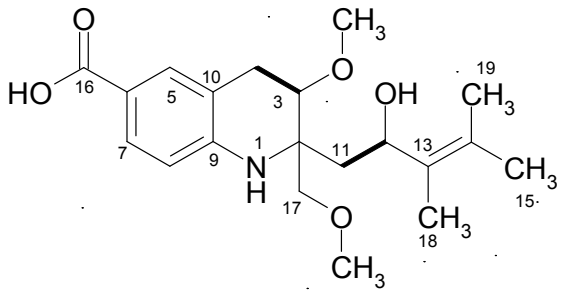
Indole-5-carboxylic acid (43): UV absorbing, orange solid, R_f = 0.60 (cyclohexane/60% ethyl acetate), orange colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.32 (dd, ^4J = 1.5, ^5J = 0.5 Hz, 1H, H-4), 7.80 (dd, ^3J = 8.6, ^5J = 1.6 Hz, 1H, H-6), 7.41 (d, ^3J = 8.6 Hz, 1 H, H-7), 7.31 (d, ^3J = 3.2 Hz, 1H, H-2), 6.56 (dd, ^3J = 3.2, ^4J = 0.6 Hz, 1H, H-3). – ^{13}C NMR (CD_3OD, 600 MHz) δ 171.7 (CO-8), 140.4 (C_q-7a), 129.0 (C_q-3a), 127.3 (CH-2), 124.6 (CH-4), 123.8 (CH-6), 122.4 (C_q-5), 111.8 (CH-7), 103.8 (CH-3). – **EIMS** *m/z* 161 ([M^+], 100), 144 (68), 116 (48), 89 (24). – **HREIMS** ([M]$^+$, 161.04780), (calcd for $C_9H_7NO_2$, 161.04768).



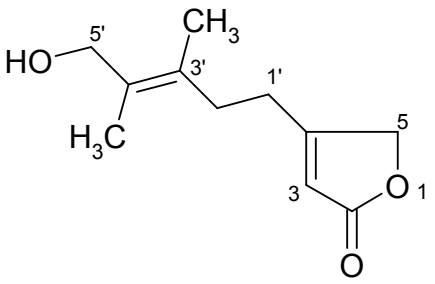
Ramthacin A (44): UV absorbing, colourless oil, dark green colouration with anisaldehyde/sulphuric acid, R_f = 0.83 (cyclohexane/60% ethyl acetate). – ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.77 (dd, ^3J = 8.3, ^4J = 1.9 Hz, 1H, H-7), 7.76 (d, ^4J = 1.9 Hz, 1H, H-5), 6.52 (d, ^3J = 8.3 Hz,1H, H-8), 5.54 (t, ^3J = 7.7 Hz, 1H, H-12), 5.02 (s, 1H, H_a-15), 4.93 (s, 1H, H_b-15), 4.23 (t, ^3J = 4.8 Hz, 1H, H-3), 3.51 (s, 2H, H-17), 3.38 (s, 3H, OCH_3-17), 3.36 (dd, ^3J = 16.9, ^2J = 4.8 Hz, 1H, H_a-4), 3.10 (dd, ^3J = 17.2, ^2J = 5.1 Hz, 1H, H_b-4), 2.67 (dd, ^3J = 14.8 ^2J = 8.5 Hz, 1H, H_a-11), 2.43 (dd, ^3J = 14.8, ^2J = 7.1 Hz, 1H, H_b-11), 1.88 (s, 3H, CH_3-19), 1.78 (s, 3H, CH_3-18). – ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.5 (CO-16), 146.8 (C_q-9), 144.2 (C_q-14), 139.1 (C_q-13), 132.5 (CH-5), 130.4 (CH-7), 119.9 (CH-12), 117.6 (C_q-6), 115.8 (C_q-10), 113.5 (CH-8), 112.4 (CH_2-15), 74.1 (CH_2-17), 59.3 (OCH_3-17), 58.8 (C_q-2), 56.4 (CH-3), 34.4 (CH_2-11), 33.5 (CH_2-4), 21.0 (CH_3-19), 13.9 (CH_3-18). – **(+)-ESIMS** *m/z* 350 ([M + H]$^+$, 90), 436 ([2 M + Na]$^+$, 100). – **(-)-ESIMS** *m/z* 348 ([M - H]$^-$, 100), 697 ([2 M - H]$^-$, 100). – **(+)-HRESIMS** *m/z* 350.15230 [M + H]$^+$, (calcd for $C_{19}H_{25}NO_3Cl$, 350.15229), 372.13423 [M + Na]$^+$, (calcd for $C_{19}H_{24}NO_3ClNa$, 372.13423).



Ramthacin B (47): UV absorbing, colourless oil, R_f = 0.73 (cyclohexane/60% ethyl acetate), dark green colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.79 (dd, ^3J = 9.1, ^4J = 1.8 Hz, 1H, H-7), 7.77 (d, ^4J = 1.8 Hz, 1H, H-5), 6.59 (d, ^3J = 9.1 Hz,1H, H-8), 4.91 (dd, ^3J = 10.0, ^3J = 2.4 Hz 1H, H-12), 4.60 (t, ^3J = 11.1 Hz,1H, H-3), 3.67 (s, 2H, H-17), 3.48 (dd, ^3J = 17.5, ^2J = 4.9 Hz, 1H, H_A-4), 3.42 (s, 1H, 3- H, OCH_3-17), 3.11 (dd, ^3J = 17.3, ^2J = 6.2 Hz, 1H, H_B-4), 2.05 (m, 1H, H_a-11), 1.74 (dd, ^3J = 14.4, ^2J = 2.4 Hz, 1H, H_b-11), 1.63 (s, 9H, CH_3-15,18,19). – **^{13}C NMR** (125 MHz, $CDCl_3$): δ 171.9 (CO-16), 146.7 (C_q-9), 132.5 (CH-5), 130.3 (CH-7), 129.7 (C_q-13), 126.6 (C_q-14), 118.2 (C_q-6), 116.5 (C_q-10), 114.1 (CH-8), 74.7 (CH_2-17), 67.3 (CH-12), 59.4 (OCH_3-17), 58.0 (C_q-2), 57.0 (CH-3), 40.3 (CH_2-11), 33.8 (CH_2-4), 21.1 (CH_3-18), 19.5 (CH_3-15), 12.1 (CH_3-19). – (+)-ESIMS *m/z* 801 ([2 M – 2 H + 3 Na]$^+$, 50), 412 ([M - H + 2 Na]$^+$, 100). – **(-)-ESIMS** *m/z* 755.4 ([2 M – 2 H + Na]$^-$, 100), 733.2 ([2 M - H]$^-$, 70), 366.0 ([M - H]$^-$, 10). – **EIMS** *m/z* 367 ([M]$^+$, 10), 322 (12), 304 (40), 258 (100), 224 (68), 99 (34). – **HREIMS** *m/z* 367.15500 [M] $^+$, (calcd for $C_{19}H_{26}NO_4Cl$, 367.15503).



Ramthacin C (48): UV absorbing, colourless oil, R_f = 0.62 (cyclohexane/60% ethyl acetate) dark green with anisaldehyde/sulphuric acid. – **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.78 (dd, ^3J = 8.3, ^4J = 1.8 Hz, 1H, H-7), 7.74 (d, ^4J = 1.8 Hz, 1H, H-5), 6.57 (d, ^3J = 8.3 Hz,1H, H-8), 4.88 (dd, ^3J = 9.7, ^3J = 2.1 Hz, 1H, H-12), 3.66 (t, ^3J = 6.6 Hz,1H, H-3), 3.65 (s, 2H, H-17), 3.42 (s, 3-H, OCH_3-3), 3.40 (s, 3H, OCH_3-17), 3.12 (dd, ^3J = 17.0, ^2J = 5.1 Hz, 1H, H_a-4), 2.85 (dd, ^3J = 16.7, ^2J = 6.7 Hz, 1H, H_B-4), 1.97 (dd, ^3J = 14.8, ^3J = 10.0 Hz, 1H, H-11$_b$), 1.68 (m, 1H, H_b-11), 1.63 (s, 6H, 2 CH_3, CH_3-18,19), 1.60 (s, 3H, CH_3-15). – **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.8 (CO-16), 147.5 (C_q-9), 132.7 (CH-5), 130.1 (CH-7), 130.1 (C_q-13), 125.9 (C_q-14), 117.8 (C_q-6), 117.2 (C_q-10), 114.1 (CH-8), 76.3 (CH-3), 74.3 (CH_2-17), 66.8 (CH-12), 59.4 (OCH_3-17), 56.9 (OCH_3-3), 41.2 (CH_2-11), 27.5 (CH_2-4), 21.1 (CH_3-18), 19.5 (CH_3-15), 12.1 (CH_3-19). – (+)-ESIMS *m/z* 408 ([M - H + 2 Na]$^+$, 100), 386 ([M + Na]$^+$, 34). – **(-)-ESIMS** *m/z* 725 ([2 M - H]$^-$, 100), 362 ([M – H]$^-$, 78). – **(+)-ESIMS** *m/z* 408 ([M –H + Na]$^+$, 100), 386.2 ([M + Na]$^+$, 38). – **HRESIMS** *m/z* 386.19450 [M + Na] $^+$, (calcd for $C_{20}H_{29}NO_5Na$, 386.19433).



4-(5-Hydroxy-3,4-dimethyl-pent-3-enyl)-5*H*-furan-2-one (MR2521ga) (49): UV absorbing, colourless oil, no colour reaction with anisaldehyde/sulphuric acid. – ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.87 (s, 1H, H-5), 4.76 (d, ^{2}J = 1.7 Hz, 2H, H-3), 4.15 (s, 2H, H-5'), 2.52 (m, 2H, H-1'), 2.39 (m, 2H, H-2'), 1.77 (s, 6H, CH_3-3',4'). – ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9 (CO-1), 169.9 (C_q-4), 130.2 (C_q-3'), 129.9 (C_q-4'), 73.1 (CH_2-3), 63.7 (CH_2-5'), 32.3 (CH_2, C-2'), 26.2 (CH_2-1'), 17.6 (CH_3-3'), 16.2 (CH_3-4). – (+)-ESIMS *m/z* 219 ($[M + Na]^+$, 100), 415, ($[2 M + Na]^+$, 20).

9.5 Marine *Streptomyces* sp. B6924

The marine *Streptomyces* sp. B6924 was cultivated on agar plates for three days at 28 °C and exhibited a white aerial mycelium. The plate was used to inoculate a 2 L shaker culture. The brownish culture broth was extracted with ethyl acetate and the resulting crude extract used for different activity tests.

9.5.1 Pre-Screening

The biological screening of the crude extract showed good antibacterial activity as shown in Table 19.

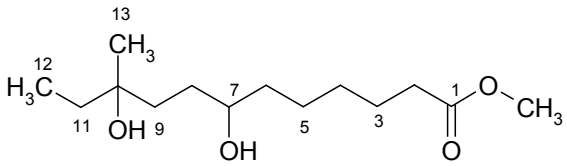
Table 19. Antimicrobial activity of the crude extract from marine *Streptomyces* sp. B6924.

Tested- microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	20
Staphylococcus aureus	18

9.5.2 Fermentation and Isolation

The marine derived *Streptomyces* sp. B6924 was precultivated on M_2 50% seawater medium agar plates at 28 °C for 3 days. With pieces of well-grown agar subculture of the strain, 30 L Shaker culture were cultivated at 28 °C with 250 rpm for 9 days. The well-grown culture broth was mixed with Celite and filtered *in vacuo*. The mycelium and filtrate were separately extracted with ethyl acetate and through XAD-16, respectively; the organic phases showed a similar composition and were combined and evaporated under vacuum to dryness. The oily brownish crude extract (2.2 g) was chromatographed on silica gel using a stepwise CH_2Cl_2/MeOH gradient to yield four

fractions. Further purification of fraction III and IV using silica gel column and Sephadex LH-20 afforded five known compound: actinomycin D (5.7 mg), niax (**67**, 5.2 mg), N-acetyltyramine (1.7 mg), deoxyuridine and thymine, as well as one new compound, 7,10-dihydroxy-10-methyldodecanoic acid methyl ester (**53**, 3.3mg).



7,10-Dihydroxy-10-methyl-dodecanoic acid methyl ester (53): Colourless oil, R_f = 0.30 (CH_2Cl_2/5% MeOH), no UV absorption, no colour reaction with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 3.64 (s, 3H, OCH_3), 3.53 (m, 1H, H-10), 2.33 (t, ^3J = 7.4 Hz, 2H, H-2), 1.60 (m, 2H, H-3), 1.48 (m, 2H, H-11), 1.42 (m, 1H, H_a-6), 1.40 (m, 2H, H-8), 1.38 (m, 1H, Hb-6), 1.51-1.35 (m, 6H, H-4,5,9), 1.11 (s, 3H, CH_3-10), 0.88 (t, ^3J = 7.3 Hz, 3H, H-12). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 176.0 (CO-1), 73.5 (C_q-10), 72.2 (CH-7), 52.0 (OCH_3), 42.2 (CH_2-9), 39.1 (CH_2-8), 38.0 (CH_2-6), 35.0 (CH_2-12), 34.8 (CH_2-2), 26.3 (CH_3-10), 26.1 ($2CH_2$-3,4), 21.1 (CH_2-5), 8.5 (CH_3-12). – (+)-ESIMS *m/z* 542 ($[2\ M + Na]^+$, 65), 283 ($[2\ M + Na]^+$, 100). – **HRESIMS** *m/z* 261.20672 (calcd for $C_{14}H_{29}O_4$, 261.20657).

9.6 Marine *Streptomyces* sp. B8041

The marine derived *Streptomyces* sp. B8041 showed white mycelial colonies when cultivated on M_2^+ agar medium for 3 days at 28 °C.

9.6.1 Pre-screening

Well grown agar plates of the marine *Streptomyces* sp. isolate B2621 were cultivated on M_2^+ medium as shaker culture for 3 days at 28 °C. The crude extract exhibited no UV active zone in moderate polar region, gave on TLC a green colour reaction with anisaldehyde/sulphuric acid and heating. In the biological pre-screening, the crude extract revealed bioactivity as shown in Table 20.

Table 20. Antimicrobial activity of the crude extract from marine *Streptomyces* sp. B8041 (M_2^+-medium), (1 mg/ml, diameter of inhibition zones in mm).

Tested Microorganisms	Inhibition zone ∅ [mm]
Staphylococcus aureus	10

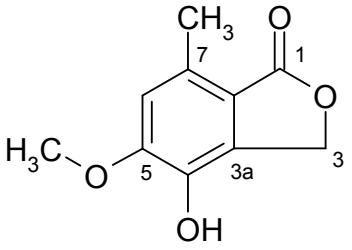
The crude extract showed 100% activity against the *Artemia salina*.

9.6.2 Fermentation and working up

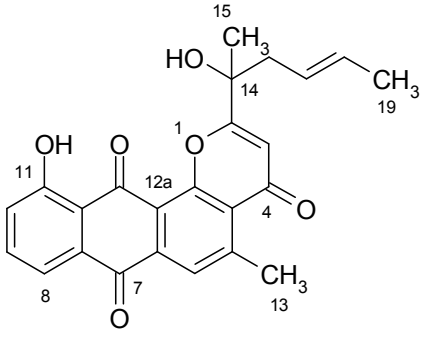
Well grown agar plates of the marine *Streptomyces* sp. isolate B2621 were used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 mL M_2^+ medium (with 50% sea water) for 5 days at 28 °C. The strain was harvested and the biomass was separated from the water phase using a filter press. The water phase was subjected to XAD-16 column and the eluate was extracted with methanol. The biomass was extracted exhaustively with ethyl acetate and acetone. The two extracts were evaporated *in vacuo* to afford brown gums, which were combined due to similar composition based on TLC.

9.6.3 Isolation

The crude extract () was subjected to silica gel column chromatography (3 × 60 cm) and eluted with CH_2Cl_2/MeOH (2.0 L CH_2Cl_2, 1.0 L CH_2Cl_2/ 1 % MeOH, 1.0 L CH_2Cl_2/ 3% MeOH, 1.0 L CH_2Cl_2/ 5% MeOH, 1.0 L CH_2Cl_2/10% MeOH, 1.0 L CH_2Cl_2/ 20% MeOH, 1.0 L CH_2Cl_2/ 50% MeOH, 0.5 L MeOH) to afford four fractions after monitoring by TLC. Fraction III was chromatographed over Sephadex LH-20 column and eluted with methanol to give three sub-fractions. Sub-fraction IIIa was further purified by PTLC to give 4-hydroxy-5-methoxy-7-methy-3*H*-isobenzofuran-1-one (**56**, 2.2 mg), *β*-indomycinone (**57**, 2.8 mg) and saptomycin A (**58**, 2.0 mg). Fraction IIIb was further purified on a silica gel column eluted with cyclohexane/ethyl acetate (60:40), which resulted in MR2621C (**61**, 4.7 mg) and a mixture (10.4 mg) of *cyclo*(isoleucyl-prolyl) (**59**), and *cis-cyclo*(prolyl-valyl) (**60**). Finally purification of IIIc led to N_β-acetyltryptamin (Figure 54).



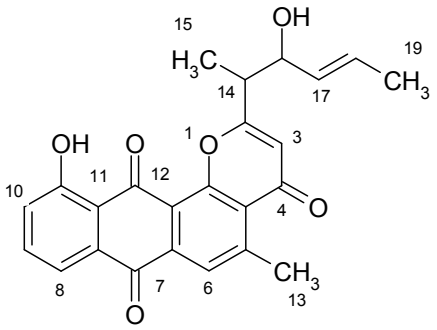
4-Hydroxy-5-methoxy-7-methy-3H-isobenzofuran-1-one (56): UV absorbing, yellow solid, R_f = 0.47 (CH_2Cl_2/3% MeOH), no colour reaction with anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz,) δ 6.77 (s, 1H, CH-6), 5.7 (brs, 1H, OH-4), 5.22 (s, 2H, CH_2-3), 3.98 (s, 3H, OCH_3-5), 2.61 (s, 3H, CH_3-7), ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.2 (C_q-1), 150.2 (C_q-5), 137.4 (C_q-4), 132.1 (C_q-3a), 132.0 (C_q-7), 116.2 (C_q-7a), 113.4 (CH-6), 66.6 (CH_2-3), 56.5 (OCH_3-5), 16.9 (CH_3-7). – **(-)-ESI MS**: *m/z* 193 ([M - H]$^-$, 100), 387 ([2 M - H]$^-$, 12). – **(-)-HRESIMS** *m/z* 193.05007 [M-H]$^-$, (calc for $C_{10}H_9O$, 193.05008).



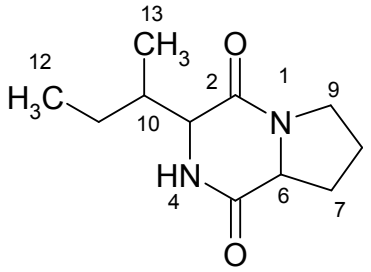
***β*-Indomycinone (57)**: Orange UV fluorescent, yellowish-orange solid, R_f = 0.78 ($CHCl_3$/5% MeOH), gives a red colour with NaOH, and turns brown with sulphuric acid. – **^{1}H**

NMR ($CDCl_3$, 300 MHz) δ 12.85 (s br, 1H, OH), 8.08 (s, 1H, H-6), 7.83 (dd, ^3J = 7.6, ^4J = 1.3 Hz, 1H, H-8), 7.68 (t, ^3J = 8.0 Hz, 1H, H-9), 7.38 (dd, ^3J = 8.4, ^4J = 1.3 Hz, H-10), 6.56 (s, 1H, H-3), 5.74 (m, 1H, H-18), 5.39 (m, 1H, H-17), 3.02 (s, 3H, CH_3-13), 2.91 (dd, ^3J = 14.4, ^3J = 8.5 Hz, 1H, H_a-16), 2.78 (dd, ^3J = 14.4, ^3J = 7.2 Hz 1H, H_b-16), 1.68 (s, 3 H, CH_3-15), 1.65 (dd, ^3J = 6.8, ^4J =1.7 Hz, 3H, CH_3-19). – **(-)-ESIMS** *m/z* 403 ([M -H]$^-$, 52), 829 ([2 M – 2 H + Na]$^-$, 90).

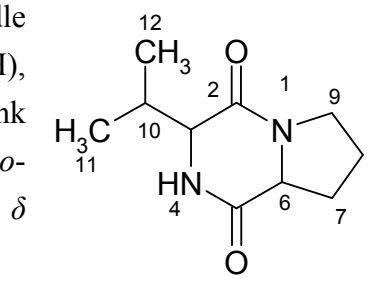
Saptomycin A (58): Orange UV fluorescent, yellow solid, R_f = 0.70 ($CHCl_3$/5% MeOH), turned to red with NaOH, and to brown with sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 12.64 (sbr, 1H, OH), 8.09 (s, 1H, H-6), 7.83 (dd, ^3J = 7.6 Hz, ^4J = 1.3 Hz, 1H, H-8), 7.69 (t, ^3J = 8.0 Hz, 1H, H-9), 7.38 (dd, ^3J = 8.4, ^4J = 1.3 Hz, 1H, H-10), 6.28 (s, 1H, H-3), 5.65 (m, 1H, H-18), 5.53 (td, ^3J = 10.5, ^3J = 6.8 Hz, 1H, H-17), 5.01 (dd, ^{3}J = 8.5, ^3J = 3.8 Hz, 1H, H-16), 3.02 (s, 3H, CH_3-13), 2.99 (m, 1H, H-14), 1.71 (dd, ^3J = 6.9, ^4J =1.7 Hz, 3H, CH_3-19), 1.45 (d, ^3J = 7.0, Hz, 3H, CH_3-15). – (+)-ESIMS *m/z* 831 ([2 M + Na]$^+$, 100). – **(-)-ESIMS** *m/z* 403 ([M - H]$^-$, 32).



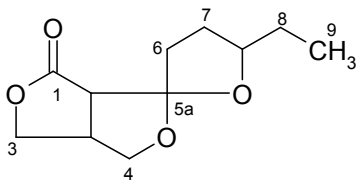
***Cyclo*(isoleucyl-prolyl) (59)**: UV absorbing, colourless solid as a middle polar substance, R_f = 0.66 (CH_2Cl_2/7% CH_3OH), turned to violet, pink, blue by anisaldehyde/sulphuric acid, Ehrlich's reagent and chlorine/*o*-anisidine reaction, respectively. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 4.19 (m, 1H, H-6), 4.07 (t, ^3J = 2.2 Hz, 1H, H-3), 3.55 (m, 2H, CH_2-9), 2.05 (m, 1H, CH_2-8), 2.13 (m, 1H, CH-10), 1.94 (m, 3H, H-8, CH_2-7), 1.46 (m, 1H, CH_2a-11), 1.30 (m, 1H, CH_2b-11), 1.06 (d, ^3J = 7.4 Hz, 3H, CH_3-13), 0.92 (t, ^3J = 7.4 Hz, 3H, CH_3-12). – ^{13}C/APT NMR (CD_3OD, 125 MHZ) δ 171.3 (C_q-5), 166.2 (C_q-2), 60.0 (CH-3), 58.7 (CH-6), 44.8 (CH_2-9), 35.7 (CH-10), 28.2 (CH_2-7), 24.1 (CH_2-11), 21.9 (CH_2-8), 14.2 (CH_3-13), 11.3 (CH_3-12). – **(-)-HRESIMS** *m/z* 209.12904 [M - H]$^-$, (calcd for $C_{11}H_{17}N_2O_2$, 209.12900).



***Cic-cyclo*(prolyl-valyl) (60)**: UV absorbing, middle polar colourless solid, R_f = 0.66 (CH_2Cl_3/7% CH_3OH), turned to violet with anisaldehyde/sulphuric acid, pink with Ehrlich's reagent and gave a blue chlorine/*ortho*-anisidine reaction. – **^{1}H NMR** (CD_3OD, 300 MHz) δ



4.19 (m, 1H, H-6), 4.03 (t, ^3J = 2.1Hz, 1H, H-3), 3.53 (m, 2H, CH_2-9), 2.48 (m, 1H, 10-CH), 2.05 (m, 1H, CH_2-8), 1.94 (m, 3H, H-8, CH_2-7), 1.09 (d, ^3J = 7.7 Hz, 3H, CH_3-11), 0.92 (d, ^3J = 6.7 Hz, 3H, CH_3-12). – ^{13}C/APT NMR (CD_3OD, 125 MHz) δ 171.1 (C_q-5), 166.2 (C_q-2), 60.2 (CH-3), 58.7 (CH-6), 44.8 (CH_2-9), 28.5 (CH-10), 28.2 (CH_2-7), 21.9 (CH_2-8), 17.5 (CH_3-11), 15.3 (CH_3-12).



(MR2621C) (61): non UV absorbing, colourless oil, R_f = 0.61 (CH_2Cl_2/3% MeOH), showed a green colour reaction with anisaldehyde/sulphuric acid. – ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.52 (t, ^3J = 9.2, 1H, H_a-3), 4.15 (m, 1H, H_a-4), 4.12 (m, 1H, H_b-3), 4.03 (m, 1H, CH-7a), 3.82 (m, 1H, H_b-4), 3.34 (m, 1H, CH-3a), 3.16 (d, ^3J = 9.0, 1H, CH-5b), 2.52 (m, 1H, H_a-6), 2.11 (m, 1H, H_a-7), 1.97 (m, 1H, H_b-6), 1.78 (m, 1H, H_b-7), 1.61 (m, 1H, H_a-8), 1.51 (m, 1H, H_b-8), 0.91 (t, ^3J = 7.5 Hz, 3H, H-9). – ^{13}C/APT NMR ($CDCl_3$, 125 MHz) δ 175.5 (C_q-1), 114.5 (C_q-5a), 82.3 (CH-7a), 73.2 (CH_2-3), 71.2 (CH_2-4), 51.6 (CH-3b), 39.3 (CH, C-3a), 32.5 (CH_2-6), 30.0 (CH_2-8), 29.8 (CH_2-7), 10.1 (CH_3-9). **– DCI:** 213 $[M + H]^+$, 230 $[M + NH_4]^+$, 425 $[2 M + H]^+$, 442 $[2 M + NH_4]^+$. **– (+)-HRESIMS** *m/z* 213.11277 $[M + H]^+$, (calcd for $C_{11}H_{17}O_4$, 213.11268).

9.7 Marine-derived *Streptomyces* sp. B7576

The marine-derived *Streptomyces* sp. B7576 was grown on M_2 agar with a white aerial mycelium after four days of incubation at 28 °C. The well-grown agar culture was used to inoculate a 1 L shaker culture in M_2^+ medium with 50% seawater, where it grew with light brown broth after six days at 28 °C with 110 rpm. The culture broth was extracted ethyl acetate by the resulting extract was used for pre-screening.

9.7.1 Pre-screening

The brown crude extract exhibited a high activity only against *Mucor miehei* (Tü 284) and weak activity against *Chlorella vulgaris, Chlorella sorokiniana* Table 21.

Table 21. Antimicrobial activity of the crude extract produced by strain B 7576.

Tested microorganisms	Inhibition zone ∅ [mm]
Mucor miehei (Tü 284)	36
Chlorella vulgaris	11
Chlorella sorokiniana	11

9.7.2 Fermentation and Isolation of metabolites

The strain B7576 was cultivated on a 25 L scale as linear shaking culture with M_2^+ at 28 °C for six days. The brown culture broth was filtered *in vacuo*. The water phase was subjected to XAD-16 followed by washing with demineralised water and at the last extracted with methanol to yield an oily crude extract A. The mycelium part was exhaustively extracted with ethyl acetate followed by acetone to give a brown extract B. TLC of both crude extracts showed no similarities and workup was done for the water phase extract. The crude extract A (5.4 g) was subjected to CC on silica gel and eluted with CH_2Cl_2/MeOH (0–40%, gradient) to yield four fractions. Fraction II was re-chromatographed over a Sephadex LH-20 column (MeOH) followed by silica gel using CH_2Cl_2/MeOH to afford niax (**67**, 12.3 mg) and a diastereomer of 3,4-dihydroxy-3-methylpentan-2-one (**68**, 21.6 mg). Further purification of fraction III and IV resulted to tyrosol, thymidine and uracil (Figure 70).

3,4-Dihydroxy-2-methylpentan-2-on (diastereomer I) (68): Non UV absorbing, colourless oil, R_f = 0.49 (CH_2Cl_2/10% CH_3OH), greenish-brown colour after spraying with anisaldehyde/sulphuric acid and heating. – ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.04 ($_q$, ^3J = 6.4, Hz, 1H, H-4), 2.29 (s, 3H, CH_3-1), 1.26 (d, ^3J = 6.4 Hz, 3H, CH_3-5), 1.25 (s, 3H, CH_3-3). – ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.7 (CO-2), 81.2 (C_q-3), 70.9 (CH-4), 23.9 (CH_3-1), 21.4 (CH_3-6), 16.7 (CH_3-5). **– DCIMS** *m/z* 167 ($[M + NH_3 + NH_4]^+$, 12), 150 ($[M + NH_4]^+$, 100).

3,4-Dihydroxy-2-methylpentan-2-one (diastereomer II) (68): Non UV absorbing, colourless oil, R_f = 0.49 (CH_2Cl_2/10% CH_3OH), greenish-brown colour after spraying with anisaldehyde/sulphuric acid and heating. – ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.87 (q, ^3J = 6.4, Hz, 1H, 4-H), 2.25 (s, 3H, CH_3-1), 1.42 (s, 3H, CH_3-3), 1.08 (d, ^3J = 6.4 Hz, 3H, CH_3-5). – ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.4 (CO-2), 81.4 (C_q-3), 71.2 (CH-4), 25.1 (CH_3-1), 21.7 (CH_3-6), 17.7 (CH_3-5). **– DCIMS** *m/z* 167 ($[M + NH_3 + NH_4]^+$, 12), 150 ($[M + NH_4]^+$, 100).

9.8 Marine-derived *Streptomyces* sp. B 8073

The marine strain *Streptomyces* sp. B 8073 was cultivated on agar plates for three days at 28 °C and exhibited a white aerial mycelium. The plate was used to inoculate a 2 L shaker culture. The brownish culture broth was extracted with ethyl acetate and the resulting crude extract used for different activity tests.

9.8.1 Pre-screening

The TLC of the resulting faint yellow extract revealed numerous middle polar UV absorbing bands, some of them showed brown, yellow colour reaction by spraying with anisaldehyde/ sulphuric acid. Moreover, the crude extract was tested for antimicrobial activity; the results are presented in Table 22.

Table 22. Antimicrobial activity of the crude extract produced by strain B 8073.

Test Test organisms	Inhibition zone ∅ (mm)
Bacillus subtilis	25
Staphylococcus aureus	22
Mucor miehei	18
Escherichia coli	28

The crude extract showed 17.5% activity against *Artemia salina*.

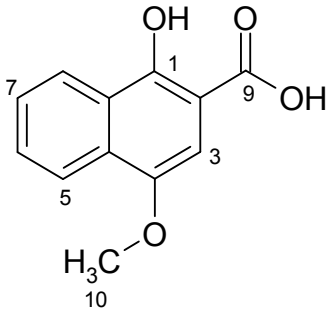
9.8.2 Fermentation

Well grown agar plates of the strain were cut into small pieces to inoculate 100 of 1-L Erlenmeyer flasks each containing 250 mL of M_2^+ medium (with 50% artificial sea water) of pH 7.8 as shaker culture (at 28 °C for 7 days). The resulting culture broth was mixed with Celite and filtered under vacuum. The water phase was subjected to Amberlite XAD-16 column with MeOH/H_2O and the methanolic phase was evaporated *in vacuo*, then the remaining water phase, was extracted with ethyl acetate (3 ×1 L). The biomass was extracted three times with ethyl acetate and acetone. As thin layer chromatography of the crude extracts of the water phase and the biomass showed similarity, both were combined.

9.8.3 Isolation and structure elucidation

The crude extract (1.2 g) of the strain *Streptomyces* sp. B 8073 was chromatographed over a silica gel column eluted using dichloromethane-methanol gradient (1-40%) to provide three fractions. Fraction II was separated by column chromatography (CC) on Sephadex LH-20 eluted with methanol to give two sub-fractions. Fraction IIa was further purified again by Sephadex LH-20 eluted with methanol to give 1-hydroxy-4-methoxy-2-naphthoic acid (**69**, 2.3 mg). Fraction III was subjected to Sephadex LH-20 (MeOH), to afford two sub-fractions. Fraction IIIb was further chromatographed by PTLC with CH_2Cl_2/5%CH_3OH, to give 20 mg staurosporine (**70**, 3.3mg).

1-Hydroxy-4-methoxy-2-naphthoic acid (69): UV absorbing, amorphous solid, R_f = 0.63 (CH_2Cl_2/5% MeOH). – ^{1}H NMR (CD_3OD, 300 MHz): δ 8.25 (dd, ^3J = 8.0, ^4J = 2.0, Hz, 1H, H-8), 8.11 (dd, ^3J = 8.1, ^4J = 2.0 Hz, 1H, H-5), 7.48 (ddd, ^3J = 6.9, ^4J = 1.2 Hz, 2H, H-6, H-7), 7.29 (s, 1H, H-3), 3.95 (s, 3H, OCH_3). – (-)-ESIMS *m/z* 217 ([M - H]$^-$, 100), 457 ([2 M – 2H + Na]$^-$, 60).



Staurosporine (70): UV absorbing, white crystals, R_f = 0.23 (CH_2Cl_2 /5% MeOH), stained to brown colour by anisaldehyde/sulphuric acid. – ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.29 (d, ^3J = 7.9 Hz, 1H, H-4), 7.98 (t, ^3J = 7.7 Hz, 2H, 8-H, H-11), 7.57 (d, ^3J = 8.2 Hz, 1H, H-1), 7.43 (m, 2H, H-2, 10), 7.28 (m, 2H, H-3,9), 6.70 (dd, ^3J = 3.9, ^3J = 2.4 Hz, 1H, H-6'), 4.95 (s, 2H, CH_2-7), 4.07 (d, ^3J = 3.2 Hz, 1H, H-3'), 3.30 (s, 3H, OCH_3-3'), 3.28 (m, 1H, CH-4'), 2.50 (m, 2H, CH_2-5'), 2.31 (s, 3H, CH_3-2'), 1.53 (s, 3H, NCH_3). – (+)-ESIMS *m/z* (%) 933 ([2 M + H]$^+$, 30), 467 ([M + H]$^+$, 100).

9.9 Marine derived *Streptomyces* sp. MRB8013

The marine derived *Streptomyces* sp. isolate B8013 was pre-cultivated on M_2 50% seawater at 28 °C for 3 days and showed a white mycelium, they were used to inoculate a 2 L shaker culture on rotary shaker (95 rpm) at 28 °C for 7 days. The brown culture broth was extracted with ethyl acetate and yielded a brown oily crude extract.

9.9.1 Primary screening

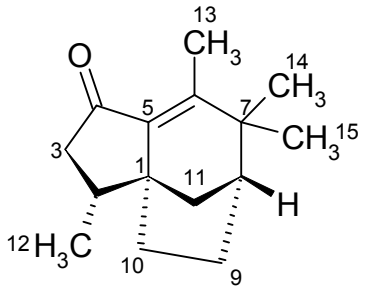
The antimicrobial test by agar diffusion showed the results as presented in the Table 23 below. The chemical screening on TLC showed non-UV absorbing zones, which developed a violet colour with anisaldehyde/sulphuric acid. The most interesting spots were blue fluorescent under UV light at 366 nm and showed blue-green colouration with anisaldehyde/sulphuric acid. In addition, many other zones were only visible after spraying with anisaldehyde/sulphuric acid as violet spots, which turned red after a few minutes.

Table 23. Biological activity of the crude extract of the MRB8013

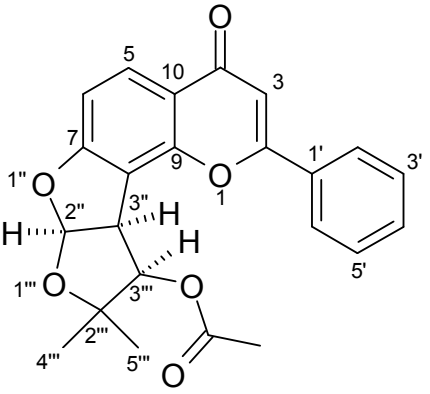
Test Test organisms	**Inhibition zone ∅ (mm)**
Mucor miehei	15
Streptomyces aureus	11
Candida albicans	19

9.9.2 Fermentation, Extraction and Isolation

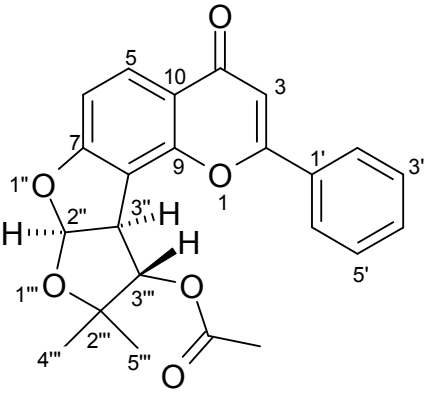
With pieces of well grown agar subculture of the strain, 100 of 1 L Erlenmeyer flasks each containing 300 ml of M_2 100% seawater medium were inoculated and cultivated at 28 °C with 95 rpm for seven days. The well-grown culture was mixed with ca. 1.5 kg Celite and filtered by pressure filtration. The mycelium and filtrate were separately extracted with ethyl acetate; the organic phases showed a similar composition and were combined and evaporated to dryness. The oily residue 3.5 g was chromatographed on silica gel using CH_2Cl_2/0-50% MeOH gradient to yield nine fractions. Purification of fraction FI on silica gel with cyclohexane/dichloromethane (50:50) followed by Sephadex L-20 eluted with MeOH led to the isolation of albaflavenone (**71**, 2.1 mg). Separation on Sephadex L-20 (MeOH) of FIII gave two sub fractions FIIIa and FIIIb, which were further purified by RP18 (MeOH/H_2O 40:60) to afford semiglabrin (**73**, 0.45 mg), *p*-hydroxybenzoic acid methyl ester (**74**, 1.4 mg) and 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**87**, 1.5 mg). Fraction IV was purified in the same method as FIII to give pseudosemiglabrin (**72**, 1.8 mg) and antimycin A-complex (**75**, 5.4 mg). Purification of fraction FV on Sephadex LH-20 MeOH followed by reverse phase RP18 afforded 2,5-bis(3-indolylmethyl)pyrazine (**76**, 1.4 mg) and MNK-003B (**77**, 1.6 mg). Sephadex LH-20 (MeOH) for FVI gave indole-3-carboxylic acid and a mixture of 4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**78**, 1.6 mg) and 4,9,10-trihydroxy-10-methyldodec-2-en-1,4-olide (**79**, 4.7 mg), which was further purified on RP18. Fraction VII was chromatographed over Sephadex LH-20 followed by RP18 to afford 2′-O-methyluridin (**81**, 2.2 mg), α-acetylamino-β-(3-indole)propanol (**82**, 1.8 mg) and nicotinic acid (**83**, 0.97 mg).



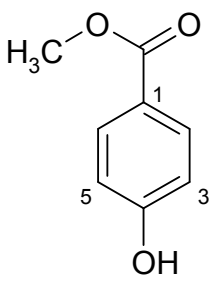
Albaflavenone: (71): Non UV absorbing, colourless oil, R_f = 0.74 (CH_2Cl_2), red colouration with anisaldehyde/sulphuric acid. − **^{1}H NMR** ($CDCl_3$, 300.0 MHz) δ 2.37 (dd, ^2J = 6.9, ^3J = 16.8 Hz, 1H, H_a-3), 2.15 (m, 1H, H-2), 2.06 (s, 3H, H-13), 2.04 (m, 1H, H_b-3), 1.88 (m, 1H, H-8), 1.80-1.30 (m, 6H, H-9, H-10, H-11), 1.12 (s, 3H, H-14), 1.09 (s, 3H, H-15), 1.03 (d, ^3J = 6.6 Hz, 3H, H-12). − ^{13}C NMR ($CDCl_3$, 125 MHz) δ 207.3 (C_q, C-4), 153.0 (C_q, C-6), 138.9 (C_q, C-5), 51.9 (C_q, C-1), 47.1 (CH_2-3), 46.2 (CH-8), 42.7 (C_q, C-7), 37.0 (CH_2, C-11), 33.3 (CH-2), 29.6 (CH_2-10), 28.4(CH_3-14), 24.5 (CH_3-15), 24.4 (CH_2-9), 14.2 (CH_3-12), 13.0 (CH_3-13). − (+) ESIMS *m/z* 459 ([2 M + Na]$^+$, 241 ([M + Na]$^+$.



Pseudosemiglabrin (72): UV absorbing, colourless solid, R_f = 0.43 (CH_2Cl_2/5 % MeOH), blue-green colouration with anisaldehyde/sulphuric acid. − ^{1}H NMR ($CDCl_3$, 300.0 MHz) δ 8.18 (d, ^3J = 8.6 Hz, 1H, H-5), 7.83 (dd, ^3J = 7.9, ^4J = 1.8 Hz, 2H, H-2', H-6'), 7.56 (dd, ^3J = 7.9, ^4J = 1.7 Hz, 2H, H-3',5'), 7.55 (m, 1H, H-4'), 6.96 (d, ^3J = 8.6 1H, H-6), 6.78 (s, 1H, H-3), 6.52 (d, ^3J = 6.5 Hz, 1H, H-2"), 5.58 (d, ^3J = 8.8 Hz, 1H, H-3'"), 4.63 (dd, ^3J = 8.8, ^3J = 6.5 Hz, 1H, H-3"), 1.49 (s, 3H, OAc-3'"), 1.40 (s, 3H, H-4'"), 1.15 (s, 3H, H-5'"). − ^{13}C NMR ($CDCl_3$, 150 MHz) δ 177.7 (C_q-4), 169.8 (CO-3'"), 164.6 (C_q-7), 162.7 (C_q-2), 153.8 (C_q-9), 131.8 (CH, C-4'), 131.3 (C_q-1'), 129.1 (CH-3', CH-5'), 128.7 (CH-5), 126.2 (CH-2', CH-6'), 118.3 (C_q-10), 111.7 (CH-2"), 111.4 (C_q-8), 109.0 (CH-6), 107.5 (CH-3), 84.6 (C_q-2'"), 77.0 (CH-3'"), 47.9 (CH-3"), 27.6 (CH_3-4'"), 23.2 (CH_3-5'"), 20.3 ($COCH_3$-3'"). − (+)-ESIMS *m/z* 806 ([2 M + Na]$^+$, 100), 415 ([M + Na]$^+$, 5). –(+)-HRESIMS *m/z* 393.13381 (calcd for $C_{23}H_{21}O_6$, 339.13381).



Semiglabrin (73): UV absorbing, colourless solid, R_f = 0.33 (CH_2Cl_2/5% MeOH), showed blue-green colour reaction with anisaldehyde/sulphuric acid. − ^{1}H NMR ($CDCl_3$, 300.0 MHz) δ 8.17 (d, ^3J = 8.6 Hz, 1H, H-5), 7.91 (m, 2H, H-2',6'), 7.56 (m, 2H, H-3',5'),), 7.55 (m, 1H, H-4'), 6.94 (d, ^3J = 8.6 1H, H-6), 6.79 (s, 1H, H-3), 6.64 (d, ^3J = 6.5 Hz, 1H, H-2"), 5.65 (s, 1H, H-3'"), 4.30 (dd, ^3J = 6.3, ^3J = 0.5 Hz, 1H, H-3"), 2.24 (s, 3H, OAc-3'"), 1.33 (s, 3H, CH_3-4'"), 1.10(s, 3H, CH_3-5'"). – EIMS (70 eV) *m/z* 392 ([M]$^{+\cdot}$, 72), 332.4 (100), 317 (60), 289 (25), 263 (52), 43 (45). – (+)-HRESIMS *m/z* 393.13381 (calcd for $C_{23}H_{21}O$, 339.13381).



***p*-Hydroxybenzoesäuremethylester (74):** UV absorbing, colourless solid, R_f = 0.63 (CH_2Cl_2/10% MeOH), no colouration with anisaldehyde/sulphuric acid.– ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92 (d, ^3J = 8.5 Hz, 2H, H-2, H-6), 6.84 (d, ^3J = 8.5 Hz, 2H, H-3, H-5), 3.83 (s, 3H, OCH3). – EIMS (70 eV) *m/z* 152 (([M]$^{\cdot+}$, 44), 121 ([M - OCH_3]$^{\cdot+}$,100), 93 ([M - $COOCH_3$]$^{\cdot+}$, 18), 65(10).

Antimycin A-complex (75): Strong blue fluorescent, colourless oil, R_f = 0.36 (CH_2Cl_2/2% MeOH), no colour reaction with anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz,) δ 12.66 (s, 1H, OH-2'), 8.56 (d, ^3J = 8.2 Hz, 1H, H-4'), 8.52 (d, ^3J = 1.2 Hz, 1H, HCO), 7.98 (br s, 1H, NH-3'), 7.24 (d, ^3J = 8.3 Hz, 1H, H-6'), 7.17 (d, ^3J = 7.6 Hz, 1H, N-3H), 6.90 (t, ^3J = 8.2 Hz, 1H, H-5'), 5.78 (quintet, ^3J = 7.2 Hz, 1H, H-4), 5.38 (t, ^3J = 7.6 Hz, 1H, H-3), 5.10 (m, 1H, H-8), 4.99 (m, 1H, H-9), 2.50 (m, 1H, H-7), 2.42 (m, 1H, H-2''), 1.80-0.80 (m, CH_2 and CH_3 for the residue R^1 and R^2). – **(+)-ESIMS** *m/z* 585 ($[M + Na]^+$, 50), 571 ($[M + Na]^+$, 100), 557 ($[M + Na]^+$, 55), 543 ($[M + Na]^+$, 5). – **(-)-ESIMS** *m/z* 561 ($[M - H]^-$, 50), 547 ($[M - H]^-$, 100), 533 ($[M - H]^-$, 40), 519 ($[M - H]^-$, 3).

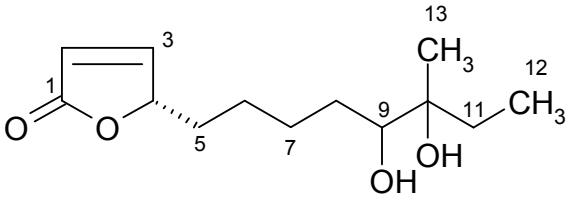
2,5-Bis(3-indolylmethyl)pyrazine (76): VU absorbing, R_f = 0.22 ($CHCl_3$/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz) δ 8.38 (s, 2H, H-3,6), 7.38 (d, ^3J = 7.9 Hz, 2H, H-7',7''), 7.33 (d, ^3J = 8.1 Hz, 2H, H-4',4''), 7.09 (s, 2H, H-2',2''), 7.07 (t, ^3J = 8.1 Hz, 2H, H-6',6''), 6.94 (t, ^3J = 7.8 Hz, 2H, H-5',5''), 4.25 (s, 4H, CH_2-8',8''). – **EIMS** (70 eV) *m/z* (%) 338 $[M]^+$ (90), 221 (11), 208 (5), 169 (8), 154 (9), 130 (100). – **(+)-ESIMS** *m/z* 699 ($[2 M + Na]^+$, 100), 361 ($[M + Na]^+$, 10), 339 ($[M + H]^+$, 5). – **(-)-ESIMS** *m/z* 675 ($[2 M - H]^-$, 10), 337 ($[M - H]^-$, 70). – **(+)-HRESIMS** *m/z* 339.16092 (calcd for $C_{22}H_{19}N_4$, 339.16096).

MNK-003B (77): colourless solid, R_f = 0.29 (CH_2Cl_2/5% MeOH), violet colour reaction with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.46 (dd, ^3J = 5.7, ^4J = 1.5 Hz, 1H, H-3), 6.12 (dd, ^3J = 5.7, ^4J = 2.0 Hz, 1H, H-2), 5.06 (m, 1H, H-4), 1.90-1.30 (m, 10H, CH_2-5,6,7,8,9), 1.22 (s, 6H, CH_3-10). – **(+)-ESIMS** *m/z* 235 ($[M + Na]^+$)

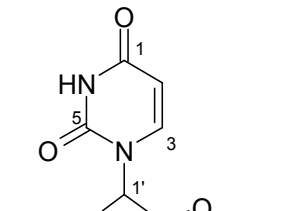
4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide (78): colourless solid, R_f = 0.29 (CH_2Cl_2/5% MeOH), violet

colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300.0 MHz) δ 7.71 (dd, ^3J = 5.7, ^4J = 1.5 Hz, 1H, H-3), 6.11 (dd, ^3J = 5.7, ^4J = 2.0 Hz, 1H, 2-H), 5.14 (m, 1H, H-4), 3.55 (q, ^3J = 6.5 Hz, 1H, H-11), 1.90-1.30 (m, 10H, CH_2-5,6,7,8,9), 1.11 (d, ^3J = 6.5 Hz, 3H, CH_3-12), 1.07 (s, 3H, CH_3-13). – **(+)-ESI MS** *m/z* 506 ([2 M + Na]$^+$, 35), 265 ([M + Na]$^+$, 100). – **(-)-ESI MS** *m/z* 483 ([2 M - H]$^-$, 80), 241.4 ([M - H]$^-$, 75).

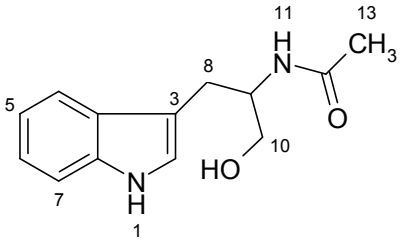
4,9,10-Trihydroxy-10-methyldod–ec-2-en-1,4-olide: (79): colourless oil, R_f = 0.20 (CH_2Cl_2/5% MeOH), blue colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (300.0 MHz, CD_3OD) δ 7.72 (dd, ^3J = 5.7, ^4J = 1.4 Hz, 1H, H-3), 6.12 (dd, ^3J = 5.7, ^4J = 2.0 Hz, 1H, H-2), 5.15 (m, 1H, H-4), 3.30 (m, 1H, H-9), 1.90-1.30 (m, 10H, CH_2-5,6,7,8,11), 1.06 (s, 3H, CH_3-10), 0.91 (t, ^3J = 7.5, 3H, CH_3-12). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 175.8 (C_q-1), 159.6 (C_q-3), 121.6 (CH-2), 85.5 (CH-4), 78.1 (CH-9), 75.5 (C_q-10), 34.1 (CH_2-5), 31.7 (CH_2-11), 31.6 (CH_2-8), 27.7 (CH_2-7), 26.1 (CH_2-6), 21.2 (CH_3-13), 7.8 (CH_3-12). – **(+)-ESIMS** *m/z* 265 ([M + Na]$^+$, 100). – **HRESIMS** *m/z* 265.14096 (calcd for $C_{13}H_{22}O_4Na$, 265.14104).



2'-O-Methyluridine (81): colourless solid, R_f = 0.29 (CH_2Cl_2/5% MeOH), green colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300.0 MHz) δ 8.08 (d, ^3J = 8.1 Hz, 1H, H-3), 5.93 (d, ^3J = 3.6 Hz, 1H, -H-1'), 5.68 (d, ^3J = 8.1 Hz, 1H, H-2), 4.23 (t, ^3J = 5.8 Hz, 1H, H-3'), 3.96 (m, 1H, H-4'), 3.87 (dd, ^3J = 12.1, ^2J = 2.5 Hz, 1H, H_a-5'), 3.84 (dd, ^3J = 8.1, ^3J = 3.6 Hz 1H, H-2') 3.73 (dd, ^3J = 12.1, ^2J = 2.9 Hz, 1H, Hb-5') 3.51 (s, 3H, OCH_3-2'). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 166.2 (C_q-1), 152.2 (C_q-5), 142.4 (CH-3), 102.5 (CH-2), 88.9 (CH-1'), 86.1 (CH-4'), 85.1 (CH-2'), 69.8 (CH-3'), 61.1 (CH_2-5'), 58.8 (OCH_3-2'). – **(+) ESIMS** *m/z* 561 ([2 M – H +2 Na]$^+$, 100), 281 ([M + Na]$^+$, 25). – (-)-ESIMS *m/z* 303 ([M - COO]$^-$, 100), 257 ([M - H]$^-$, 60). – **HRESIMS** *m/z* 257.07748 (calcd for $C_{10}H_{13}N_2O_6$, 257.07736).

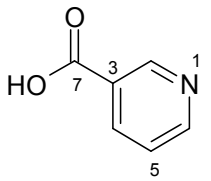


α-Acetylamino-β-(3-indole)propanol (82): colourless solid, gave a red colour reaction with anisaldehyde/sulphuric. – **^{1}H NMR** (CD_3OD, 300.0 MHz) δ 7.60 (ddd, ^3J = 7.8, ^4J = 2.0, ^5J = 1.0 Hz, 1H, H-4), 7.30 (ddd, ^3J = 7.9, ^4J = 1.8, ^5J



= 0.8 Hz, 1H, H-7), 7.08 (m, 1H, H-6), 7.06 (s, 1H, H-2), 6.99 (m, 1H, H-5), 4.19 (m, 1H, H-9), 3.54 (dd, ^3J = 6.0, ^2J = 5.2 Hz, 2H, CH_2-10), 3.01 (dd, ^3J = 14.5, ^2J = 6,8 Hz, 1H, H_a-8), 2.89 (dd, ^3J = 14.5, ^2J = 6,8 Hz, 1H, Hb-8), 1.9 (s, 3H, H-13). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 173.2 (CO-12), 138.1 (C_q-7a), 129.1 (C_q-3a), 124.0 (CH-2), 122.2 (CH-6), 119.6 (CH-5), 119.4 (CH-4), 112.3 (C_q-3), 112.1 (CH-7), 64.2 (CH_2-10), 53.6 (CH-9), 27.6 (CH_2-8), 22.8 (CH_3-13). – **(+)-ESIMS** *m/z* 487 ([2 M + Na]$^+$, 35), 255 ([M + Na]$^+$,100). – **(-)-ESIMS** *m/z* 277 ([M - COO]$^-$, 100), 231 ([M - H]$^-$, 87). ─ **HRESIMS** *m/z* 233.12907 (calcd for $C_{13}H_{17}N_2O_2$, 233.12900).

Pyridine-3-carboxylic acid; Nicotinic acid (83): UV absorbing, colourless solid, R_f = 0.45 ($CHCl_3$/10% MeOH), turned to faint pink by anisaldehyde/sulphuric after heating. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 9.02 (s, 1 H, H-2), 8.68 (d, ^3J = 4.1 Hz, 1 H, H-6), 8.28 (ddd, ^3J = 8.0, ^4J = 1.7, ^4J = 1.8 Hz, 1H, H-4), 7.54 (dd, ^3J = 5.0, 4.9 Hz, 1H, 5-H). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 169.8 (C_q-7), 152.8 (CH-6), 149.5 (CH-2), 137.3 (CH-4), 131.5 (C_q-3), 125.1 (CH-5).



9.10 Terrestrial *Streptomyces* sp. GT 2005/ 014

The terrestrial *Streptomyces* sp. GT 2005/ 014 grew on M_2 agar a with white aerial mycelium and brown pigmentation in the agar after three days of incubation at 28 °C. The strain was cultured in 4 of 1 L Erlenmeyer flasks each containing 250 ml M_2 medium on a round shaker with 95 rpm for 72 h at 28 °C, which yielded 120 mg of dark reddish-brown crude extract for the biological and chemical pre-screening.

The biological screening of this crude extract using agar diffusion method, revealed antibacterial activities, which were summarized in Table 24.

Table 24. Antimicrobial activity of the crude extract of Terrestrial *Streptomyces* sp. GT 2005/ 014.

Tested microorganisms	Inhibition zone ∅ [mm]
Streptomyces viridochromogenes (Tü57)	11
Mucor miehei (Tü 284)	15

The crude extract showed 92% activity against *Artemia salina*

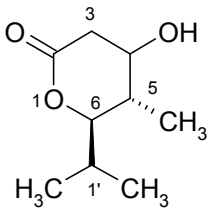
9.10.1 Fermentation and Work-up

For scaling up, the strain was cultivated on a 30 L scale at 28 °C for 8 days on a linear shaker. The brown culture broth was mixed with 1 kg diatomaceous earth and then filtered using a filter press. The water phase was adsorbed on XAD-16, the resin

washed with demineralised water (10 L) followed by methanol (15 L). The methanolic phase was evaporated *in vacuo* to the remaining water phase, which was extracted with ethyl acetate (3 ×1 L). The solid phase was extracted with ethyl acetate (3 ×1 L) followed by acetone (3×1L). The filtrate was passed through the XAD-16 column and mycelium was separately extracted four times with ethyl acetate (2 L). During the extraction of the mycelial cake, it was treated with ultrasonic radiation for 15 min each time. The yellow organic solutions were combined and the solvent was removed under vacuum resulting in 3.0 g of dark yellowish brown extract. Due to a similar composition of both extracts on TLC, they were combined to afford 3.29 g.

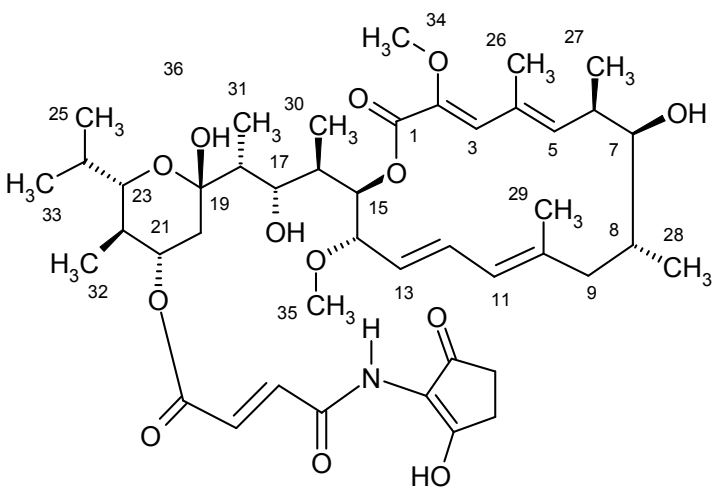
9.10.2 Isolation

The crude extract of the terrestrial *Streptomyces* sp. GT 2005/014, was fractionated over a silica gel column (CH_2Cl_2/CH_3OH gradient 0-50% MeOH) to give four fractions. Fraction II was subjected to Sephadex LH-20 (MeOH) to give three subfractions, which were re-chromatographed over a silica gel column, followed by RP 18 to give prelactone B (**84**, 2.4 mg), bafilomycin B_1 (**85a**, 1.6 mg), bafilomycin B_2 (**85b,** 0,9 mg), 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**86**) and 4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide (**87,** 1.1 mg). By the same method, nonactic acid (**88**, 4.3 mg) and (+)-homononactic acid (**93,** 3.2 mg) were isolated from fraction IV. Fraction III was further purified by Sephadex LH-20 eluted with methanol to afford indole-3-carboxylic acid, ferulic acid (**89,** 5.1 mg) and attiamycin B (**90,** 1.2 mg).



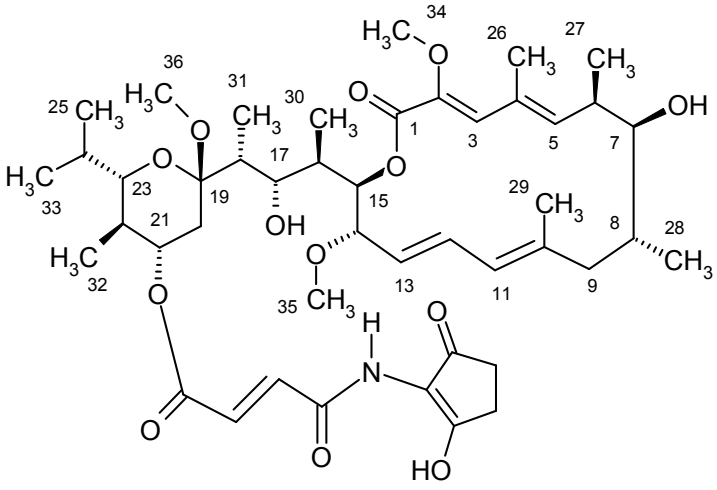
Prelactone B (84): Colourless oil, R_f = 0.38 (ethyl acetate /hexane 2:1). – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.77 (m, 2 H, H-4,6), 2.92 (dd, ^2J = 5.8, ^3J = 17.2 Hz, 1H, H_a-3), 2.49 (dd, ^2J = 7.9, ^3J = 17.7 Hz, 1H, H_b-3), 2.01 (m, 1H, H-1'), 1.76 (m, 1H, H-5), 1.10 (d, ^3J = 7.0 Hz, 3H, CH_3-1'), 1.08 (d, ^3J = 6.7 Hz, 3H, CH_3-5), 0.93 (d, ^3J = 6.3 Hz, 3 H, CH_3-2'). – **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.1 (CO-2), 86.3 (CH-6), 69.7 (CH-4), 39.0 (CH-5), 38.9 (CH_2-3), 28.9 (CH-1'), 20.0 (CH_3-2'), 14.0 (CH_3-5), 13.6 (CH_3-1'). – **(+)-ESIMS** *m/z* 173 ($[M + H]^+$, 10), 195 ($[M + Na]^+$, 55), 367 ($[2 M + Na]^+$, 100). – **(+)-HRESIMS** 173.11788 ($[M + H]^+$ (calcd for $C_9H_{17}O_3$,173.11776).

Bafilomycin B_1 (85a): UV absorbing, yellow amorphous powder, R_f = 0.31 (CH_2Cl_2/5% MeOH), gave a reddish-green colour reaction by spraying with anisalde-



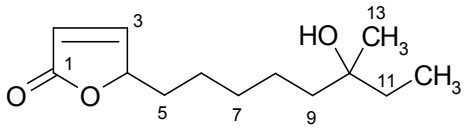
hyde/sulphuric acid and heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 13.51 (s br, 1H, OH), 9.18 (s br, 1H, NH), 7.25 (d, ^3J = 15.1 Hz, 1H, H-39), 6.89 (d, ^3J = 15.5 Hz, 1H, H-38), 6.70 (d, ^4J = 0.7 Hz, 1H, H-3), 6.57 (dd, ^3J = 15 Hz, 10.5, 1H, H-12), 5.82-5.80 (td, ^3J = 10.5 Hz, ^4J =1.2 Hz, 2H, -H-5,11), 5.60 (s br, 1H, OH), 5.18 (dd, ^3J = 15.1 Hz, ^4J = 9.4 Hz, 1H, H-13), 5.16 (ddd, ^3J = 11.0, 9.9, 4.7 Hz, 1H, H-21), 4.98 (dd, ^3J = 8.7, 1.2 Hz, 1H, H-15), 4.70 (s br, 1H, OH), 4.18 (d br, ^3J = 7.1 Hz, 1H, H-17), 3.91 (dd, ^3J = 9.5, 8.7 Hz, 1H, H-14), 3.62 (s, 3H, H-34), 3.61 (m, 1H, H-7), 3.32 (d br, ^3J = 5.3 Hz, 1H, H-23), 3.22 (s, 3H, H-35), 2.64 (m, 4H, $2CH_2$), 2.57 (m, 1H, H-6), 2.40 (dd, ^3J = 11.9, 4.7 Hz, 1H, -H-20a), 2.18 (dm, ^3J = 14.0 Hz, 1H, H-9a), 2.13 (m, 1H, H-16), 1.99 (s, 3H, H-26), 1.96 (dd, ^3J = 14.0, 11.5 Hz, 1H, H-9b), 1.95 (s, 3H, H-29), 1.92 (m, 1H, H-8), 1.79 (q br, ^3J = 7.1 Hz, 1H, H-24), 1.63 (m, 1H, H-18), 1.28 (m, 1H, H-22), 1.17 (m, 1H, H-20a), 1.08 (d, ^3J = 7.0 Hz, 3H, CH_3-27), 1.03 (d, ^3J = 7.2 Hz, 3 H, CH_3-31), 0.95 (d, ^3J = 6.5 Hz, 3H, CH_3-32), 0.94 (d, ^3J = 6.5 Hz, 3H, CH_3-28), 0.92 (d, ^3J = 6.7 Hz, 3 H, CH_3-25), 0.83 (d, ^3J = 6.7 Hz, 3H, CH_3-30), 0.78 (d, ^3J = 6.7 Hz, 3H, CH_3-33). – **(-)-ESIMS** *m/z* 814 $[M - H]^-$. – **(+)-ESIMS** *m/z* 838 ($[M + Na]^+$, 89), 860 ($[M - H + 2 Na]^+$).

Bafilomycin B_2 (85b): UV absorbing, yellow solid, R_f = 0.34 (CH_2Cl_2/5% MeOH), gave a reddish-green colour after spraying with anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.88 (s br, 1H, NH), 7.05 (d, ^3J = 15.2 Hz, 1H, H-39), 6.90 (d, ^3J = 15.2 Hz, 1H, H-38), 6.63 (d, ^4J = 0.7 Hz, 1H, H-3), 6.57 (dd, ^3J = 15.5, 10.5 Hz, 1H, H-12), 5.83-5.79 (m, 2H, H-5,11), 5.20 (dd, ^3J = 15.1 Hz, ^4J = 9.4 Hz, 1H, H-13), 5.10-5.02 (m, 2H, H-15,21), 4.18 (dd, ^3J = 14.3, 7.2 Hz, 1H, H-17), 3.89 (dd, ^3J = 9.5 Hz, 8.7 Hz, 1H, H-14), 3.70 (s, 3H, H_3-34), 3.42 (d br, ^3J = 14 Hz, 1H, H-7), 3.23 (s, 3H, H_3-35), 3.22 (dd, ^3J = 10.2, 2.2 Hz, 1H, H-23), 3.06 (s, 3H, H_3-36), 2.64 (br, s, 4H, $2CH_2$), 2.57 (m, 1H, H-6), 2.31 (dd, ^3J = 13.2, 9.1 Hz, 1H, H-20a), 2.16 (m, 1H, H-9a), 2.12 (m, 1H, H-16), 1.99 (s, 3H, H_3-26), 1.96 (dd, ^3J = 14.0 Hz, 11.5 Hz, 1H, H-9b), 1.94 (s, 3H, H_3-29), 1.92 (dq, ^3J = 11.5 Hz, 6.5 Hz, 1H, H-8), 1.85 (m, 1H, H-24), 1.63 (m, 1H, 18-H), 1.27 (m, 1H, H-22), 1.15 (m, 1H, H-20a), 1.06 (d, ^3J = 7.0 Hz, 3H, CH_3-27), 1.04 (d, ^3J = 7.2 Hz, 3H, CH_3-31), 1.01 (d, ^3J = 6.5 Hz, 3 H, CH_3-32), 0.94 (d, ^3J = 6.5 Hz, 3H,

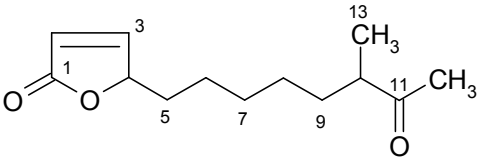


CH_3-28), 0.90 (d, ^3J = 6.7 Hz, 3H, CH_3-25), 0.89 (d, ^3J = 6.7 Hz, 3 H, CH_3-30), 0.81 (d, ^3J = 6.7 Hz, 3H, CH_3-33). – **(+)-ESIMS** *m/z* 874 ([M- H + 2 Na]$^+$, 100), 852.4 ([M + Na]$^+$, 60). – **(-)-ESIMS** *m/z* 828 [M - H]$^-$.

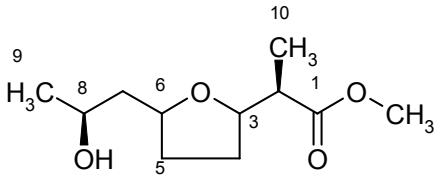
4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide (86): colourless oil, R_f = 0.53 ($CHCl_3$/5% MeOH), blue-violet with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.44 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, H-3), 6.12 (dd, ^3J = 5.6 Hz, ^4J = 2.0 Hz, 1H, H-2), 5.04 (m, 1H, 4-H), 1.78 (m, 1H, H_a-5), 1.62 (m, 1H, Hb-5), 1.50-1.20 (m, 8H), 1.12 (s, 3H, H-13) 0.90 (t, ^3J = 7.6 Hz, 3H, H-12). – (+)-ESIMS *m/z* 477.0 ([2 M + Na]$^+$, 30), 249.0 ([M + Na]$^+$, 100). – **(+)-HRESIMS** 227.16470 ([M + H]$^+$ (calcd for $C_{13}H_{23}O_3$, 277.14671).



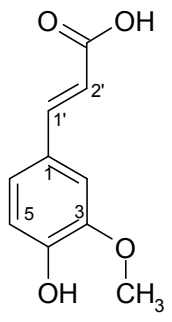
4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (87): colourless oil, R_f = 0.37 (CH_2Cl_2/5% MeOH), violet with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz) δ 7.46 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, H-3), 6.12 (dd, ^3J = 5.6 Hz, ^4J = 2.0 Hz, 1H, H-2), 5.04 (m, 1H, H-4), 2.50 (m, 1H, H-10), 2.12 (s, 3H, H-12), 1.70 (m, 1H), 1.60 (m, 2H), 1.50-1.20 (m, 7H), 1.10 (d, ^3J = 7.0 Hz, 3H, H-13). – **(+)-ESIMS** *m/z* 471 ([2 M + Na]$^+$, 48), 247 ([M + Na]$^+$, 100). – **(-)-ESIMS** *m/z* 222 ([M - H]$^-$, 15).

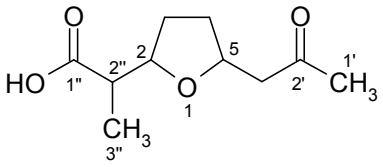


Nonactic acid (88): Non UV absorbing, colourless oil, R_f = 0.38 (CH_2Cl_2/5% MeOH), stained to violet by anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** ($CDCl_3$, 300.132 MHz) δ 7.20 (s br, 2H, 2 OH), 4.22 (m, 1H, H-6), 4.08 (m, 1 H, H-8), 3.99 (q, ^3J = 8.3 Hz, 1H, H-3), 2.50 (dq, ^3J = 8.3, 7.2 Hz, 1H, H-2), 2.02 (m, 2H, H_a-4, Ha-5), 1.68 (m, 4 H, Hb-4, Hb-5, 7-CH_2), 1.21 (d, ^3J = 6.4 Hz, 3H, H-9), 1.17 (d, ^3J = 7.2 Hz, 3H, H-10). – **(+)-ESIMS** *m/z* 225 ([M + Na]$^+$, 55), 203 ([M + H]$^+$, 31). – **(+)-HRESIMS** 203.1278010 ([M + H]$^+$ (calc for $C_{10}H_{19}O_4$, 203.12833)



Ferulic acid (89): UV absorbing, colourless solid, R_f = 0.27 (CH_2Cl_2/10 % MeOH), stained to violet with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 7.58 (d, ^3J = 15.9 1H, H-1'), 7.16 (d, ^4J = 1.9 Hz, 1H, H-2'), 7.05 (dd, ^3J = 8.2, ^4J = 2.0 Hz, 1H, H-6), 6.80 (d, ^3J = 8.1 Hz, 1H, H-5), 6,30 (d, ^3J = 15.9 Hz, 1H, H-2'), 3.88 (s, 3H, OCH_3-3). – (-)-ESIMS *m/z* 193 ([M - H]$^-$, 100).





Attiamycin B, 2-[5-(2-Oxo-propyl)-tetrahydro-furan-2-yl]-propionic acid (90): Non UV absorbing, colourless oil, R_f = 0.46 (CH_2Cl_2/ 5% MeOH), turned violet by anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.85 (br s 1H, OH), 4.28 (m, 1H, H-5), 4.06 (m, 1H, H-2), 2.80 (dd, ^3J = 16.1, ^2J = 6.7 Hz, 1H, H_a-3'), 2.55 (dd, ^3J = 15.8, ^2J = 6.7 Hz, 1H, Hb-3'), 2.52 (m, 1H, H-2''), 2.18 (s, 3H, H-1'), 2.10 (m, 1H, H_a-4), 1.90 (m, 1H, H_a-3), 1.61 (m, 1H, H_b-3), 1.53 (m, 1H, H_b-4), 1.15 (d, ^3J = 6.8 Hz, 3H, CH_3-3''). – **(+)-ESIMS** *m/z* 223 ($[M + Na]^+$, 65), 423 ($[2 M + Na]^+$, 100). – **(-)-ESIMS** *m/z* 199 ($[M - H]^-$, 100), 398 ($[2 M - H]^-$, 68).

9.11 Terrestrial *Streptomyces* sp. Gt-2005-009

The terrestrial *Streptomyces* sp. Gt-2005-009 grew with a white thick aerial mycelium on M_2 agar after incubation at 28 °C for three days. With the well grown strain on agar, eight of 1 L Erlenmeyer flasks each with 250 ml of the same medium were inoculated and grown at 28 °C on a linear shaker with 95 rpm, whereby the strain grew with light brown broth. The dark brown extract obtained from the 2 L shaker culture was used for the biological and chemical screening.

9.11.1 Primary screening

The extract was found to inhibit the growth of Gram-positive and Gram-negative bacteria, fungi and algae. The semiquantitative results of antibacterial, antifungal and phytotoxic tests are listed in the following table. The TLC of the crude extract exhibited four colourless non UV absorbing bands, which became first brown and later turned to violet with anisaldehyde/sulphuric acid. In addition, yellow bands appeared, which turned to dark orange on spraying with anisaldehyde/sulphuric acid.

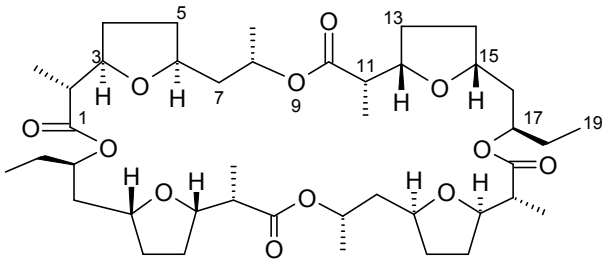
Table 25. Biological activity of the crude extract of the strain GT 2005/ 009.

Test organisms	Inhibitionzone ∅ (mm)
Bacillus subtilis	20
Mucor miehei	11
Streptomyces aureus	17
Streptomyces viridochromogenes (Tü 57)	0
Candida albicans	13
Escherichia coli	14

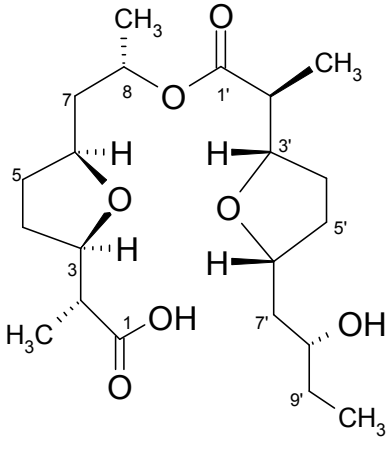
The crude extract showed 100% activity against *Artemia salina*

9.11.2 Fermentation and Work-up

The strain was cultured on a 30 L scale on a linear shaker with 95 rpm in 1 L Erlenmeyer flasks each containing 300 ml of M_2 medium at 28 °C for five days. The resulting pale brown culture broth was filtered through a filter press and the filtrate was eluted with methanol through XAD-16 column and the methanolic extract was evaporated under vacuum, the mycelium was extracted with ethyl acetate and acetone. As TLC of the three different extracts showed no differences, extracts were combined and evaporated to dryness yielding a dark brown oily material (7.07 g). Chromatography of the crude extract on silica gel using a CH_2Cl_2/MeOH gradient resulted to five fractions. The second, third and fourth fractions contained non-polar, non UV absorbing bands which showed brown and violet colour reactions on TLC after spraying with anisaldehyde/sulphuric acid. Purification of fraction I using Sephadex LH-20 (MeOH) resulted in dinactin (**91**, 100.2 mg). Fraction III was further purified by Sephadex LH-20 (MeOH) followed by silca gel CC to give bonactin (**92,** 10.5 mg). Fraction four was chromatographed on Sephadex LH-20 (MeOH) and then PTLC (CH_2Cl_2/ 7% MeOH) to give (+)-homononactic acid (**93**, 2.2 mg) (Figure 112).

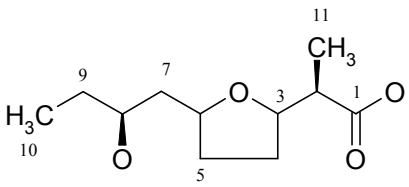


Dinactin (91): Non UV absorbing, colourless oil, R_f = 0.27 ($CHCl_3$/10% MeOH), which gave a violet band on spraying with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 4.93 (m, 2 H, H-8,17), 4.02 (br q, 2H, H-3,12), 3.87 (m, H-6,15), 2.52 (m, 2H, H-2,11), 2.01 (m, 2 H, CH_2-5,14), 1.92 (m, 2 H, CH_{2a}-4,13), 1.67 (m, 2H, CH_2-7), 1.56 (m, 2H, CH_{2b}-4,13), 1.56 (m, 2H, CH_2-18), 1.49 (m, 2H, CH_2-5,14), 1.23 (d, ^3J = 6.2 Hz, 3H, CH_3-8), 1.10 (d, ^3J = 7.0 Hz, 3 H, CH_3-11), 1.08 (d, ^3J = 7.0 Hz, 3H, CH_3-2), 0.87 (t, ^3J = 7.4 Hz, 3 H, CH_3-19). – ^{13}C NMR ($CDCl_3$, 75.476 MHz) δ 174.2 (C_q-1), 173.9 (C_q-10), 79.8 (CH-12), 79.6 (CH-3), 76.1 (CH-6), 76.0 (CH-15), 72.9 (CH-17), 68.8 (CH-8), 45.0 (CH-11), 44.8 (CH-2), 42.0 (CH_2-7), 39.7 (CH_2-16), 31.2 (CH_2-5), 31.1 (CH_2-14), 27.9 (2 CH_2-4,13), 27.1 (CH_2-18), 20.2 (CH_3, 8-CH_3), 2.9 (CH_3, 2-CH_3), 9.1 (CH_3-19). – **(+)-ESI MS** *m/z* 787 ([M + Na]$^+$, 100).



Bonactin (92): Non UV absorbing, colourless oil (13 mg), R_f = 0.27 ($CHCl_3$/10% MeOH) coloured to violet by anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.45 (s br, 3H, 3 OH), 5.08-4.87 (m, 1H, H-8), 4.18 (m, 1H, H-6'), 3.98 (m, 3H, H-3,3',6), 3.75 (m, 1H, H-8'), 2.50 (m, 2H, H-

2,2'), 2.08-1.93 (m, 4H, CH_2-5,4), 1.86-1.42 (m, 10H, CH_2-5',4',7,7',9'), 1.24 (d, ^3J = 6.4 Hz, 3H, CH_3-8), 1.17 (d, 3J = 7.2 Hz, 3H, CH_3-2), 1.13 (d, ^3J = 7.2 Hz, 3H, CH_3-2'), 0.96 (t, 3J = 6.2 Hz, H, CH_3-10'). – **(+)-ESIMS** *m/z* 423.7 ($[M + Na]^+$), 401 ($[M + H]^+$).



(+)-Homononactic acid (93): Non UV absorbing, colourless oil, R_f = 0.40 (CH_2Cl_2/5% MeOH), turns first brown and then violet with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.98 (s br, 2H, 2 OH), 4.21 (m, 1H, H-6), 3.99 (bq, ^3J = 8.3 Hz, 1H, H-3), 3.79 (m, 1H, H-8), 2.50 (dq, ^3J = 8.3, 7.0 Hz, 1H, H-2), 2.03 (m, 1H, CH_2-4), 2.01 (m, 1H,, CH_2-5), 1.70 (m, 2H, CH_2-7), 1.68 (m, 1H, CH_2-4), 1.65 (m, 1H,, CH_2-5), 1.49 (m, 2H, CH_2-9), 1.18 (d, ^3J = 7.1 Hz, 3H, CH_3-11), 0.92 (t, ^3J = 7.6 Hz, 3H, CH_3-10). – **(+)-ESIMS** *m/z* 239 ($[M + Na]^+$, 90), 455 ($[2 M + Na]^+$, 100), 477 ($[2 M –H + 2 Na]^+$, 95). – **(-)-ESIMS** *m/z* 215 ($[M - H]^-$, 100).

9.12 Terrestrial *Streptomyces* Wo 990

The terrestrial *Streptomyces* Wo 990 was sub-cultivated in M2 medium on agar plates at 28 °C for four days. Twelve of 1 L Erlenmeyer flasks each containing 250 ml of the medium were inoculated using one agar plate. The flasks were kept at 28 °C on a rotary shaker (95 rpm) for three days. The resulting broth was filtered under vacuum and both of the filtrate and the biomass were extracted with ethyl acetate to yield brown oily crude extract.

9.12.1 Pre-screening

The chemical screening of the crude extract showed two bands, which turned greenish-blue and brownish green with anisaldehyde/sulphuric acid, respectively.

Table 26. Biological activities of crude extract of the terrestrial *Streptomyces* sp. Wo 990 and compound **94**.

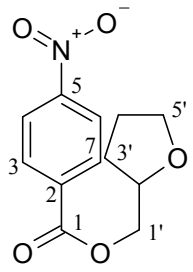
Test organisms	Inhibition zone (Ø in [mm]) of crude extract	Inhibition zone (Ø in [mm]) of Compd. 94.
Bacillus subtilis	18	0
Staphyloccus aureus	22	0
Streptomyces viridochromogenes (Tü 57)	20	0
Escherichia coli	22	0

Candida albicans	20	0
Mucor miehei (Tü 284)	14	0
Chlorella vulgaris	0	23
Chlorella sorokiniana	11	34
Scenedesmus subspicatus	0	30

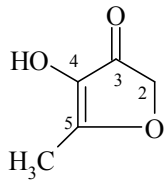
The crude extract was showed 90% cytotoxicity against brine shrimp and compound **94** showed 54%.

9.12.2 Fermentation, Work-up and Isolation

The strain was cultivated at 28 °C for seven days using a linear shaker. 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated with agar cultures. The dark brown culture broth was mixed with 1.2 kg diatomaceous earth and filtered under vacuum. The biomass was extracted three times with ethyl acetate (2 L) followed by acetone. The filtrate was subjected to XAD-16 column, the resin washed with distilled water. The combined extracts were concentrated under vacuum to obtain a crude extract (4 g). This material was subjected to silica gel column chromatography (CC) eluting with CH_2Cl_2 followed by stepwise addition of CH_3OH to yield four fractions. Fraction II was subjected to Sephadex LH-20 CC eluted with CH_3OH followed by reverse phase RP-18 eluted with MeOH/water to afford 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**94**, 3.3 mg) and 4-Hydroxy-5-methylfuran-3-one (**98**, 2.4 mg). Fractions III and IV were purified in the same way of fraction II to delivered uridine, phenylalanine and methylthioadenosine.



4-Nitrobenzoic acid tetrahydrofuran-2-yl-methyl-ester (94): UV absorbing, colourless solid, m.p. 45-8 °C, R_f = 0.75 (CH_2Cl_2/1% MeOH), green colouration with anisaldehyde/sulphuric acid. $[\alpha]_D^{20}$ -1.2 (c 1.00, MeOH). – **^{1}H NMR** (300 MHz, CD_3OD) and – **^{13}C NMR** (125 MHz, CD_3OD) see Table 11. – **(-)-ESIMS** *m/z* (%) 520 ($[2\ M + NH_4]^+$, 15), 252 ($[M + H]^+$, 20), 269 ($M + NH4]^+$, 100). – **ESIHRMS** *m/z* $[M + H]^+$ (calcd: for $C_{12}H_{13}NO_5$, 252.08679); 252.08665 $[M + Na]^+$ (calcd for $C_{12}H_{13}NO_5Na$, 274.06872).



4-Hydroxy-5-methylfuran-3-one (98): Coulourless solid, R_f = 0.64 (CH_2Cl_2/ 2% MeOH), brownish-green colouration with anisaldehyde/sulphuric acid. – ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 11. – **EIMS** (70 eV) *m/z* 114 ($[M]^+$, 75).

9.13 Terrestrial *Streptomyces* Ank 150

The terrestrial S*treptomyces* sp. Ank 150 was cultivated on M_2 agar plates for three days at 28 °C. The plates were used to inoculate a 1 L culture using M_2 medium during four days at 28 °C. The brown culture broth was worked up at standard conditions and the resulting crude extract was used for biological activity tests.

9.13.1 Pre-screening

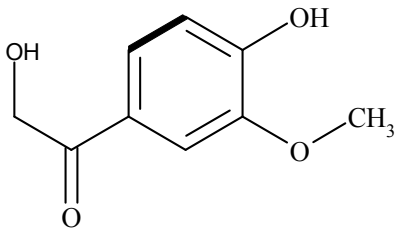
Chemical screening by TLC revealed two UV absorbing bands stained to greenish-blue and reddish-brown with anisaldehyde/sulphuric acid and heating. In the biological screening, the crude extract exhibited the activities shown in Table 27.

Table 27. Biological activities of crude extract of the Terrestrial *Streptomyces* Ank 150.

Test organisms	Inhibition zone Φ [mm]
Bacillus subtilis	11
Staphyloccus aureus	10
Streptomyces viridochromogenes (Tü 57)	15
Escherichia coli	10

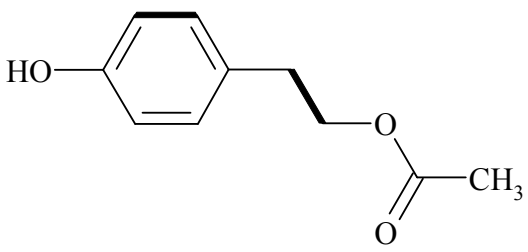
9.13.2 Fermentation, Extraction and Isolation

The terrestrial *Streptomyces* strain Ank150 was cultivated in the same way on M_2 medium. The obtained crude extract (2.5 g) from a 30 L shaker culture was chromatographed over a silica gel column eluted with CH_2Cl_2/MeOH of increasing polarity to afford three fractions. Fraction II and III were further purified by Sephadex LH-20 followed reverse phase RP18 to give α-hydroxyacetovanillone (**101**, 5.0 mg) and acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**102**, 3.0 mg).



α-Hydroxyacetovanillone (101): UV absorbing, colourless solid, R_f = 0.52 (CH_2Cl_2/3% MeOH), greenish-blue colouration with anisaldehyde/sulphuric acid and heating. – **UV** (MeOH) λ_{max} (log ε) 301 (3.93), 276 (4.02), 229 (4.17) 204 (4.19) nm; (MeOH/HCl): 302 (3.93), 276 (4.05), 229 (4.16), 204 (4.19); (MeOH/NaOH): 344 (4.32), 327 (4.80), 210. – **^{1}H NMR** (300 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 12. – (-)-**ESIMS** *m/z* 385 ([2 M –2 H + Na]$^-$, 100), 181.2 ([M - H]$^-$, 100). – **HRESIMS** *m/z* 183.0651660 [M + H]$^+$ (calcd for $C_9H_{11}O_4$, 183.0651853).

Acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (102): Colourless solid, R_f = 0.52 (CH_2Cl_2/3% MeOH), reddish-brown colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (600 MHz, CD_3OD) and **^{13}C NMR** (125 MHz, CD_3OD) see Table 13. **– (+)-ESIMS** *m/z* 203 $[M + Na]^+$.



9.14 Terrestrial *Streptomyces* sp. Ank 192

The strain Ank 150 gave a white aerial mycelium after incubation on M_2 medium at 28 °C for three days. One agar plate was used to inoculate 12 of 1 litre-Erlenmeyer flasks each containing 250 ml of M_2 medium. The culture was grown at 28 °C on a linear shaker with 95 rpm for five days. The dark brown crude extract obtained from the 3 L shaker culture was used for the biological and chemical screening.

9.14.1 Pre-screening

The chemical screening of the crude extract showed a band, which gave a dark green colouration with anisaldehyde/sulphuric acid.

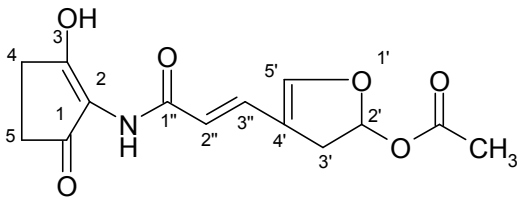
Table 28. Biological activities of crude extract of the terrestrial *Streptomyces* sp. Ank 192

Test organisms	Inhibition zone ∅ [mm]
Bacillus subtilis	12
Staphyloccus aureus	15
Escherichia coli	12

The crude extract was showed 81% activity aginst Brine shrimp.

9.14.2 Scale-up of the strain and isolation

The strain was cultured on a 30 L scale on a linear shaker with 100 rpm in 1 L Erlenmeyer flasks each containing 300 ml of M_2 medium at 28 °C for seven days. The resulting pale brown culture broth was filtered under vacuum, the biomass was extracted three times with ethyl acetate followed by acetone. The filtrate was passed through XAD-16 resin then washed with the methanol. The combined crude extracts (3.2 g) were fractionated on a silica gel column using a CH_2Cl_2/CH_3OH system to get three fractions. Fraction II was subjected to Sephadex LH-20 column chromatography (MeOH) to afford reductiomycin (**104**, 5.3 mg); another sub-fraction, which was further purified by RP18 afforded α-hydroxyacetovanillone (**101**, 1,1 mg) (Figure 138).



Reductiomycin (**104**)**:** UV absorbing, yellow needles, R_f = 0.31 ($CHCl_3$/4% MeOH), deep green colour by spraying with anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 13.76 (br s, 1 H, OH), 7.68 (br s, 1 H, NH), 7.50 (d, ^3J = 15.1 Hz, 1H, H-3''), 6.90 (s, 1H, H-5'), 6.70 (dd, ^3J = 7.5, 2.3 Hz, 1H, H-2'), 5.81 (d, ^3J = 15.1 Hz, 1H, H-2''), 3.05 (m, 1H, one of CH_2-3'), 2.60 (m, 5 H, CH_2-4,5, one of CH_2-3'). – **(+)-ESIMS** *m/z* 607 ($[2\ M - H + Na]^+$, 100), 292 ($[M - H]^+$, 77).

9.15 Terrestrial *Streptomyces* sp. Ank22

9.15.1 Pre-screening

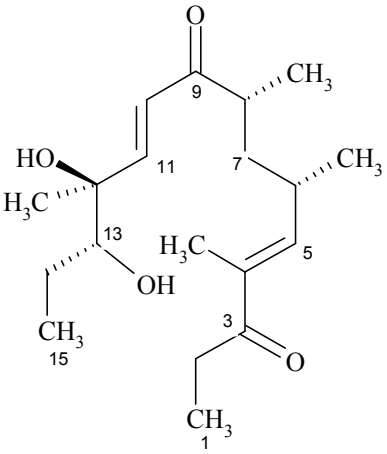
Well grown agar plates of the terrestrial *Streptomyces* sp. Ank22 were used to inoculate 1 litre of M_2 medium using a rotary shaker for 3 days at 28 °C. The TLC of the resulting faint brown extract revealed numerous polar UV absorbing bands, most of which turned to violet-pink by spraying with anisaldehyde/ sulphuric acid. The crude extract was tested for antimicrobial activity (Table 29).

9.15.2 Fermentation, Work-up and Isolation

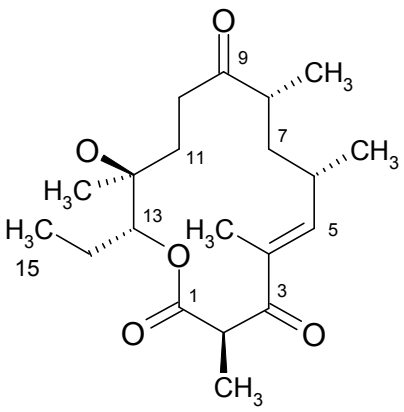
The terrestrial *Streptomyces* sp. Ank22 strain was fermented in a 30 L scale on a linear shaker for seven days at 28 °C. The resulting dark brown culture broth was mixed with Celite and filtered *in vacuo*. The water phase was passed over an XAD-16 column (65 × 8), the resin washed with distilled water and eluted with methanol, while the mycelium was extracted with ethyl acetate followed by acetone. Both crude extract were combined, based on the TLC and evaporated to dryness to afford 2.5 g of dark reddish-brown crude extract. This extract was chromatographed over a silica gel column eluted using a CH_2Cl_2/CH_3OH gradient to provide four fractions. Fraction II was separated by column chromatography (CC) on Sephadex LH-20 eluted with methanol to give two sub-fractions which were further purified by C18 column eluted with MeOH:H_2O which resulted in α-Hydroxyacetovanillone (**101**, 1.4 mg) and a mixture (8.6 mg) of 10,11-dihydro-kromycin (**106**)/kromycin (**107**). Fraction III was subjected to Sephadex LH-20 eluted with methanol to afford actinomycin D and sub-fraction IIIb which was further purified over C18 column to give *seco*-decarboxy-kromycin (**105**, 4.9 mg)). Neomethymycin (**108**, 10.0), methymycin (**109**,10.0 mg), and picromycin (**110**, 4.3 mg) were isolated from fraction IV in the same way as for **105**.

Table 29. Antimicrobial activity of the crude extract from the Ank22**.**

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	28
Escherichia coli	30
Staphylococcus aureus	20
Streptomyces viridochromogenes (Tü57)	25

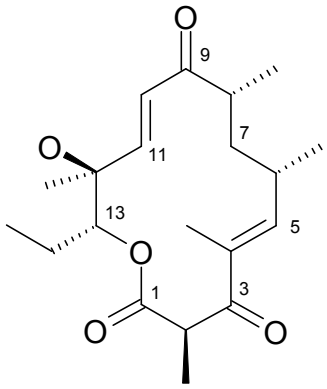


***seco*-Decarboxy-kromycin (105):** UV absorbing, colourless oil, R_f = 0.13 (CH_2Cl_2/5% MeOH), gave dark violet colour with anisaldehyde/ sulphuric acid and heating. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 6.98 (d, ^3J = 15.9 Hz, 1H, H-11), 6.40 (dd, ^3J = 10.2, ^4J = 1.4 Hz, 1H, H-5), 6.35 (d, ^3J = 15.9 Hz, 1H, H-10), 3.25 (dd, ^3J = 10.5, ^3J = 2.1 Hz, 1H, H-13), 2.84 (m, 1H, H-8), 2.69 (qd, ^3J = 7.4, ^3J = 2.3 Hz, 2H, H-2), 2.66 (m, 1H, H-6), 1.84 (m, 1H, H_a-7), 1.75 (d, ^3J = 1.4 Hz 3H, CH_3-4), 1.55 (m, 2H, H-14), 1.44 (m, 1H, H_b-7), 1.24 (s, CH_3-12), 1.07 (d, ^3J = 5.9, 3H, CH_3-8) 1.04 (t, ^3J = 3.4 3H, H-1), 1.02 (d, ^3J = 6.1, 3H, CH_3-6), 0.98 (t, ^3J = 7.4, 3H, H-15). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 206.1 (CO-9), 205.0 (CO-3), 153.1 (CH-11), 149.3 (CH-5), 136.6 (C_q-4), 127.3 (CH-10), 79.9 (CH-13), 76.3 (C_q-12), 43.6 (CH-8), 41.2 (CH_2-7), 33.6 (CH-6), 31.3 (CH_2-2), 25.4 (CH_2-14), 23.5 (CH_3-12), 20.5 (CH_3-6), 18.0 (CH_3-8), 11.5 (CH_3-15), 11.7 (CH_3-4), 9.2 (CH_3-1). – **(+)-ESIMS** *m/z* 671 ([2 M + Na]$^+$, 53), 347 ([M + Na]$^+$, 55). – **(-)-ESIMS** *m/z* 369 ([M -HCOO]$^-$, 100). – **HRESIMS** *m/z* 347.21985 [M + Na]$^+$, (calcd for $C_{19}H_{32}O_4Na$, 347.21982).

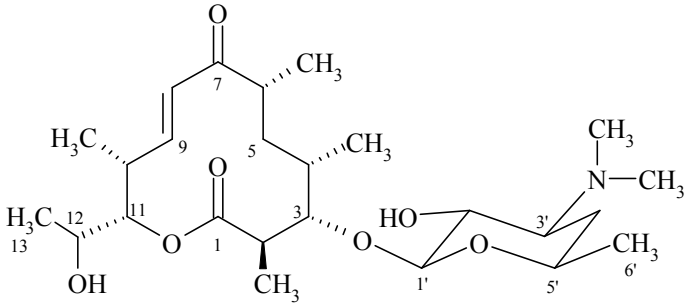


10,11-Dihydro-kromycin (106): UV absorbing, white crystals, R_f = 0.28 (CH_2Cl_2/5% MeOH), yellow-brown colour with anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.34 (dd, ^4J = 1.2, ^3J = 8.1 Hz, 1H, H-5), 4.80 (dd, ^4J = 2.4, ^3J = 10.8 Hz, 1H, H-13), 4.30 (q, ^3J = 7.0 Hz, 1H, H-2), 2.74 (m, 1H, H-6), 2.50 (m, 1H, Ha-10), 2.46 (m, 1H, H-8), 2.36 (m, 1H, H_b-10), 1.90 (d, ^4J = 1.3, 3H, CH_3-4), 1.83 (m, 1H, H_a-11), 1.71 (m, 1 H, H_a-14), 1.60 (m, 1H, H_b-11), 1.52 (m, 2H, H_a-7, Hb-14), 1.41 (d, ^3J = 6.9 Hz, 3H, CH_3-2), 1.40 (m, 1H, Hb-7), 1.19 (s, 3H, CH_3-12), 1.04 (d, ^3J = 6.6 Hz, 6H, CH_3-6,8), 0.93 (t, ^3J = 7.3 Hz, 3H, H_3-15). – **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 215.4 (CO-9), 196.1

(CO-3), 172.1 (CO-1), 147.4 (CH-5), 137.9 (C_q-4), 82.3 (CH-13), 73.6 (C_q-12), 45.6 (CH-2), 42.0 (CH-8), 40.7 (CH_2-7), 37.0 (CH-10), 32.6 (CH-6), 32.0 (CH-11), 23.4 (CH_3-12), 22.7 (CH_2-14), 20.9 (CH_3-6), 15.7 (CH_3-8), 14.3 (CH_3-2), 12.3 (CH_3-4), 10.9 (CH_3-15). – **(+)-ESIMS** *m/z* 725 ($[2\ M + Na]^+$, 50), 375 ($[M + Na]^+$, 18). – **(+)-HRESIMS** *m/z* 352.23286 $[M + H]^+$, (calcd. for $C_{20}H_{33}O_5$, 352.23279).



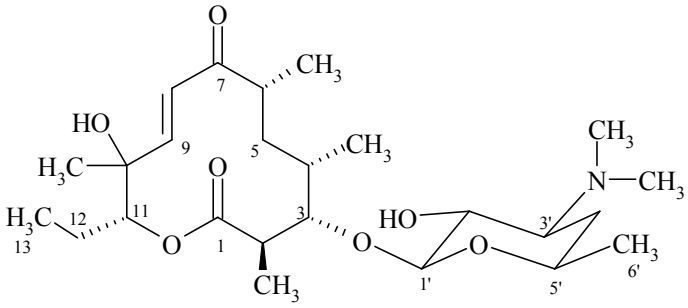
Kromycin (107): UV absorbing, white crystals (10 mg), R_f = 0.0.28 (CH_2Cl_2/5% MeOH), yellow-brown colour with anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.72 (d, ^3J = 16.6 Hz, 1H, H-11), 6.33 (dd, ^4J = 1.2, ^3J = 6.3 Hz, 1H, H-5), 6.05 (d, ^3J = 16.6 Hz, 1H, H-10), 4.86 (dd, ^4J = 2.4, ^3J = 10.9 Hz, 1H, H-13), 4.30 (q, ^3J = 7.0 Hz, 1H, H-2), 2.84 (m, 1H, H-6), 2.77 (m, 1H, H-8), 1.91 (d, ^4J = 1.3, 3H, CH_3-4), 1.84 (m, 1H, H_a-14), 1.58 (m, 1H, H_a-7), 1.50 (m, 1H, Hb-14), 1.43 (d, ^3J = 6.9 Hz, 3H, CH_3-2), 1.38 (m, 1H, Hb-7), 1.35 (s, 3H, CH_3-12), 1.06 (d, ^3J = 6.1 Hz, 3H, CH_3-8), 1.04 (d, ^3J = 6.4 Hz, 3H, 6-CH_3-6), 0.93 (t, ^3J = 7.4 Hz, 3H, CH_3-15). – **^{13}C NMR** (CDCl3, 125 MHz) δ 204.1 (CO-9), 196.3 (CO-3), 171.5 (CO-1), 149.6 (CH-11), 147.5 (CH-5), 138.2 (C_q-4), 127.4 (CH-10), 80.7 (CH-13), 74.0 (C_q-12), 45.6 (CH-2), 43.0 (CH_2-7), 39.6 (CH-8), 33.0 (CH-6), 22.1 (CH_2-14), 21.7 (CH_3-12), 21.2 (CH_3-6), 15.0 (CH_3-8), 13.8 (CH_3-2), 12.5 (CH_3-4), 10.5 (CH_3-15). – **(+)-ESIMS** *m/z* 723 ($[2\ M + Na]^+$, 100), 373 ($[M + Na]^+$, 18).



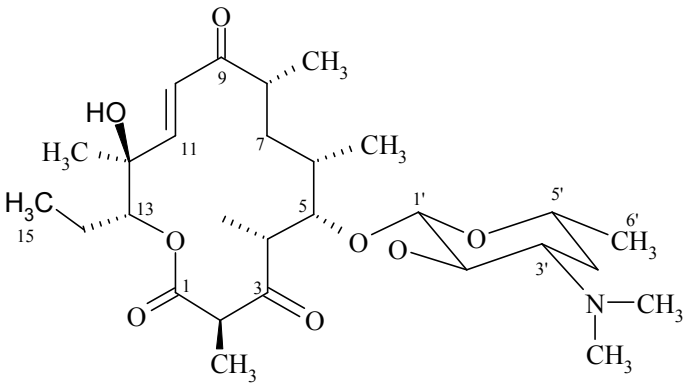
Neomethymycin (108): UV absorbing, brown oil, brown colour with anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.59 (dd, ^3J = 15.7, 5.3 Hz, 1H, H-9), 6.42 (dd, ^3J = 15.7, ^4J = 1.2, 1H, H-8), 4.78 (dd, ^3J = 9.0, 2.3 Hz, 1H, H-11), 4.32 (d, ^3J = 6.9 Hz, 1H, H-1'), 3.86 (m, 1H, H-12), 3.60 (m, 1H, H-3), 3.55 (m, 1H, H-5'), 3.51 (m, 1H, H-2'), 3.39 (m, 1H, H-3'), 3.04 (m, 1H, H-10), 2.84 (s, 6H, $N(CH_3)_2$), 2.84 (m, 1H, H-2), 2.52 (m, 1H, H-6), 2.11 (m, 1H, H_a-4'), 1.62 (m, 1H, H_a-5), 1.48 (m, 1H, H_b-4'), 1.34 (d, ^3J = 7.0 Hz, 3H, CH_3-2), 1.35 (m, 1H, Hb-5), 1.27 (d, ^3J = 6.2 Hz, 3H, H-6'), 1.24 (m, 1H, H-4), 1.18 (d, ^3J = 7.0 Hz, 3H, H-13), 1.17 (d, ^3J = 6.0 Hz, 3H, CH_3-6), 1.14 (d, ^3J = 6.8 Hz, 3H, CH_3-10), 0.96 (d, ^3J = 6.7 Hz, 3H, CH_3-4). – **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 204.9 (CO-7), 174.5 (CO-1), 147.3 (CH-9), 126.1 CH-8), 103.6 (CH-1'), 85.8 (CH-3), 75.6 (CH-11), 69.7 (CH-2'), 67.9 (CH-5'), 66.5 (CH-12) 66.4 (CH-3'),

45.1 (CH-6), 43.6 (CH-2), 40.4 ($N(CH_3)_2$), 35.5 (CH-10), 34.1 (CH_2-5), 33.4 (CH_2-4), 31.6 (CH_2-4'), 20.9 (CH-13) 20.7 (CH_3-6'), 17.7 (CH_3-6),17.4 (CH_3-4) 16.1 (CH_3-2), 9.8 (CH_3-10). – **(+)-ESIMS** *m/z* 939 ($[2\ M + H]^+$, 100), 470 ($[M + H]^+$, 55). – **HRESIMS** *m/z* 470.31180 $[M + H]^+$, (calcd for $C_{25}H_{44}NO_7$, 470.31176).

Methymycin (109): UV absorbing, brown oil, brown colour with anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.73 (dd, ^3J = 15.9 Hz, 1H, H-9), 6.32 (dd, ^3J = 15.9 Hz, 1H, H-8), 4.73 (dd, ^3J = 10.7, 2.2 Hz, 1H, H-11), 4.32 (d, ^3J = 6.9 Hz, 1H, H-1'), 3.60 (m, 1H, H-3), 3.55 (m, 1H, H-5') 3.51 (m, 1H, H-2'), 3.39 (m, 1H, H-3'), 2.84 (s, 6H, $N(CH_3)_2$), 2.84 (m, 1H, H-2), 2.54 (m, 1H, H-6), 2.11 (m, 1H, Ha-4'), 1.92 (m, 2H, H-12), 1.62 (m, 1H, H_a-5), 1.48 (m, 1H, H_b-4'), 1.35 (s, 3H, CH_3-10), 1.35 (d, ^3J = 6.1, 3H, CH_3-2), 1.34 (m, 1H, Hb-5), 1.27 (d, ^3J = 6.2 Hz, 3H, H-6'), 1.24 (m, 1H, H-4), 0.96 (d, ^3J = 6.7 Hz, 3H, CH_3-4), 0.87 (t, ^3J = 7.4 Hz, 3H, H-13). – **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 204.2 (CO-7), 175.0 (CO-1), 149.0 (CH-9), 125.5 (CH-8), 103.5 (CH-1'), 85.7 (CH-3), 76.6 (CH-11), 74.4 (C_q-10), 69.7 (CH-2'), 67.9 (CH-5'), 66.4 (CH-3'), 45.1 (CH-6), 40.4 ($N(CH_3)_2$), 34.1 (CH_2-5), 33.4 (CH_2-4), 31.6 (CH_2-4'), 22.2 (CH_2-12), 20.7 (CH_3-6'), 19.4 (CH_3-10), 17.4 (CH_3-4), 16.4 (CH_3-2), 10.7 (CH_3-13). – **(+)-ESIMS** *m/z* 939 ($[2\ M + H]^+$, 100), 470 ($[M + H]^+$, 55). – **(+)-HRESIMS** *m/z* 470.31180 $[M + H]^+$, (calcd for $C_{25}H_{44}NO_7$, 470.31176).



Picromycin (110): UV absorbing, brown oil, brown colour with anisaldehyde/sulphuric acid after heating. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.63 (d, ^3J = 15.4 Hz, 1H, H-11), 6.31 (d, ^3J = 15.4 Hz, 1H, H-10), 5.01 (d, ^3J = 11.1 Hz, 1H, H-13), 4.35 (d, 4.35 (d, ^3J = 7.3 Hz, 1H, H-1'), 3.80 (m, 1H, H-2), 3.48 (m, 1H, H-5'), 3.15 (m, 1H, H-2'), 3.11 (m, 1H, H-4) 2.62 (m, 1H, H-8), 2.45 (m, 1H, H-3'), 2.20 (s, 6H, $N(CH_3)_2$), 2.20 (m, 1H, H-6), 1.65 (m, 1H, H_a-14), 1.59 (m, 1H, H_a-4'), 1.36 (d, ^3J = 7.4 Hz, 3H, CH_3-2), 1.33 (m, 1H, H_b-14), 1.23 (s, 3H, CH_3-12), 1.18 (m, 1H, H_b-4'), 1.14 (d, ^3J =



6.2 Hz, 1H, H-6'), 1.01 (d, ^3J = 6.4, 3H, CH_3-4), 1.01 (d, ^3J = 6.4 Hz, 3H, CH_3-8), 0.95 (d, ^3J = 7.0 Hz, 3H, CH_3-6), 0.90 (m, 2H, H-7), 0.79 (t, ^3J = 7.2 Hz, 3H, CH_3-15). – **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 212.5 (CO-3), 203.7 (CO-9), 170.2 (CO-1), 145.4 (CH-11), 128.8 (CH-10), 104.8 (CH-1'), 83.1 (CH-13), 74.8 (C_q-12), 69.8 (CH-2'), 69.5 (CH-5'), 65.5 (CH-3'), 53.1 (CH-2), 46.4 (CH-4), 46.4 (CH-4), 42.7 (CH-8), 40.1 (NCH_3), 37.4 (CH_2-7), 35.7 (CH-6), 28.2 (CH_2-4'), 23.0 (CH_2-14), 22.7 (CH_3-12), 21.0 (CH_3-6'), 17.3 (CH_3-6), 14.7 (CH_3-4), 14.7 (CH_3-8), 13.2 (CH_3-2) 10.6 (CH_3-15). − **(+)-ESIMS** *m/z* 526 ($[M + H]^+$, 40). – **(-)-ESIMS** *m/z* 524 ($[M - H]^-$, 100). – **HRESIMS** *m/z* 526.33780 $[M + H]^+$, (calcd for $C_{28}H_{48}NO_8$, 526.33797).

9.16 Teresterial *Streptomyces* sp. Ank123

9.16.1 Fermentation and working up

The strain was cultivated on M_2^+ medium in the scale of 30 L for ten days. The grey culture broth was mixed with Celite and filtered through a filter press. The water phase was subjected to XAD-16 column, then washed with deionised water and eluted by methanol. The methanol was evaporated and the remaining water phase was extracted with ethyl acetate. The mycelium phase was extracted with ethyl acetate then acetone. The organic phases were brought to dryness and the crude extracts of both phases were combined based on TLC.

9.16.2 Isolation

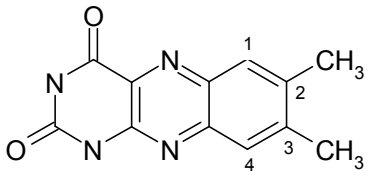
The crude extract (3.4 g) was chromatographed over a flash silica gel column to yield four fractions. Fraction II was submitted to Sephadex LH-20 (MeOH) to afford kromycin (**107**, 2.6 mg); sub-fraction IIa was further purified *via* C18 column to yield 3-butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (**113**, 1.3 mg). Fraction III was re-chromatographed on Sephadex LH-20 (MeOH) to give two sub-fractions IIIa and IIB. The latter one was further purified by RP18 to afford monensin B (**119**, 5.6 mg). Repeated chromatography of sub-fraction IIB over Sephadex LH-20 (MeOH) followed by C18 column yielded lumichrome (**111**, 1.4 mg), 6-Methoxy-6-propyl-tetrahydro-furo[3,4-c]furan-1-one (**117**, 1.2 mg) and tyrosol. Fraction IV was purified in a similar way to give indole-3-carbonyl-L-rhamnopyranoside (**112**, 2.1 mg) (Figure 156).

9.16.3 Pre-screening

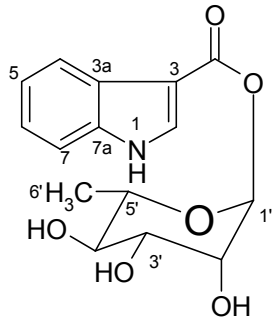
The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57) (Table 30).

Table 30. Antimicrobial activity of the crude extract from terrestrial *Streptomyces* sp. ANK123 (M_2^+-medium), (1 mg/ml, diameter of inhibition zones in mm).

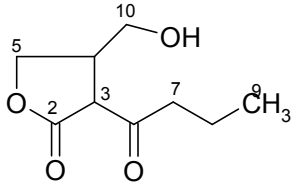
Tested Microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	10
Escherichia coli	10
Streptomyces viridochromogenes (Tü57)	20



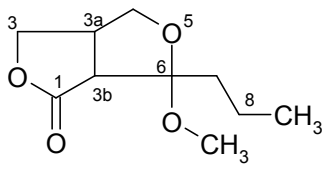
Lumichrome: (111): UV absorbing at 254 nm, colourless oil, blue fluorescence, R_f = 0.15 (CH_2Cl_2/5% CH_3OH), gave blue colouration with anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 7.95 (s, 1H, H-), 7.74 (s, 1H, H-1), 7.25 (d, ^3J = 15.1 Hz, 1H, H-4), 2.53 (s, 3H, CH_3-3), 2.50 (s, 3H, CH_3-2). – **EIMS** (70 eV) *m/z* 242 ([M]$^{+\cdot}$, 100), 171 (65), 156 (30), 144 (20).



Indole-3-carbonyl-L-rhamnopyranoside (112): UV absorbing colourless solid, R_f = 0.22 (CH_2Cl_2/10% CH_3OH), stained to orange with anisaldehyde/sulphuric. – ^{1}H NMR (CD_3OD, 300 MHz) δ 8.04 (dd, ^3J = 8.4, ^4J = 2.1 Hz, 1H, H-4), 7.46 (dd, ^3J = 8.5, ^4J = 2.3 1H, H-7), 7.22 (m, 1H, H-6), 7.21 (s, 1H, H-2), 7.20 (m, 1H, H-5), 6.21 (d, ^3J = 1.8 Hz, 1H, H-1'), 3.97 (dd, ^3J = 3.5 ^3J = 1.8 Hz, 1H, H-2'), 3.91 (dd, ^3J = 9.5, ^3J = 13.5 Hz, 1H, H-3'), 3.81 (m, 1H, H-5'), 3.51 (dd, ^3J = 9.5, ^3J = 9.5 Hz, 1H, H-4'), 1.28 (d, ^3J = 6.2 Hz, 3H, H-6'). – **(+)-ESIMS** *m/z* 636.9 ([2 M+ Na]$^+$, 100), 330.0 ([M + Na]$^+$, 55). – **(-)-ESIMS** *m/z* 613 ([2 M - H]$^-$, 100), 306.2 ([M - H]$^-$, 65). – **HRESIMS** *m/z* 308.11359 ([M + H]$^+$) (calcd for $C_{15}H_{18}NO_6$, 308.11341).



3-Butyryl-4-(hydroxymethyl)-4,5-dihydrofuran-2(3*H*)-one (113): Non UV absorbing, colourless oil, R_f = 0.63 (CH_2Cl_2/5% CH_3OH), brown colouration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 4.44 (m, 1H, H_a-5), 4.14 (m, H_b-5), 3.65 (m, 3H, H-3,10), 3.20 (m, 1H, H-4), 2.90 (m, 1H, H_a-7), 2.60 (m, 1H, Hb-7), 1.60 (m, 2H, H-8), 0.92 (d, ^3J = 7.3 Hz, 3H, H-9). – ^{13}C NMR (CD_3OD, 125 MHz) δ 202.9 (CO-6) 172.5 (CO-2), 69.1 (CH_2-5), 61.7 (CH_2-10), 54.9 (CH-3), 44.3 (CH_2-7), 39.2 (CH-4), 16.7 (CH_2-8), 13.4 (CH_3-9). – **DCIMS** *m/z* 204 ([M + NH_4]$^+$, 100).



6-Methoxy-6-propyl-tetrahydro-furo[3,4,-c]furan-1-one (117): Non UV absorbing, colourless oil, R_f = 0.27 (CH_2Cl_2/5% CH_3OH), brown coloration with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 4.44 (t, 1H, H_a-3), 4.17 (dd, ^3J = 8.9, ^3J = 8.2 Hz, 1H, H_a-4), 4.10 (dd, ^3J = 9.2, ^3J = 6.6 Hz, 1H, H_b-3), 3.78 (dd, ^3J = 9.0, ^3J = 5.4 Hz, 1H, H_b-4), 3.48 (m, 1H, H-3a), 3.25 (d, ^3J = 11.0 Hz, 1H, H-3b), 3.19 (s, 3H, OCH_3-6), 1.95 (m, 1H, H_a-7), 1.66 (m, 1H, Hb-7), 1.63 (m, 1H, H_a-8), 1.38 (m, 1H, H_b-8), 0.96 (t, ^3J = 7.4 Hz, 3H, H-9). – **^{13}C NMR** (CD_3OD, 125 MHz) δ 176.9 (CO-1), 110.7 (C_q-6), 74.1 (CH_2-3), 73.2 (CH_2-4), 56.2 (CH-3a), 48.2 (OCH_3-6), 42.8 (CH-3b), 36.3 (CH_2-7), 18.2 (CH_2-8), 14.6 (CH_3-9). – **(+)-ESIMS** *m/z* 223 ($[M + H]^+$, 50), 422 ($[2 M + H]^+$, 40). – **HRESIMS** *m/z* 223.09462 $[M + Na]^+$, (calcd for $C_{10}H_{16}O_4Na$, 223.09462).

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